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日中笹川医学奨学金制度
第 43 期＜学位取得コース＞研究者

報　告　書

2021 年 4 月～2024 年 3 月

公益財団法人　日中医学協会

日中笹川医学奨学金制度＜学位取得コース＞：第 43 期研究者

研究者No.	氏　名	所　属　機　関	受け入れ機関	指導責任者	掲載頁
		研究テーマ	学位取得方法 学位分野		
G4301	範　彬	貴州医科大学附属医院・講師	北海道大学大学院医学研究院 腫瘍病理学	田中 伸哉 教授	p. 1
		基質荷電を用いたドパミン作動性神経細胞への新規分化法の確立	課程博士 医学		
G4302	趙　雪	上海交通大学医学院附属同仁医院・主治医師	千葉大学大学院医学研究院泌尿器科学	市川 智彦 教授	p. 8
		アミノ酸トランスポーターを介した前立腺癌分子機構の解明（前列腺癌とアミノ酸トランスポーター）	課程博士 医学		
G4303	姚　利	千葉大学大学院看護学研究科・博士課程学生	千葉大学大学院看護学研究科看護学専攻	正木 治恵 教授	p. 129
		在留中国人高齢者の老いへの準備教育プログラムの開発 －ビデオカンファレンスを活用して－	課程博士 看護学		
G4304	張 茂芮	西南医科大学附属口腔医院・医師	東京医科歯科大学大学院医歯学総合研究科 生体補綴歯科学分野	若林 則幸 教授	p. 154
		骨誘導因子 Runx2 mRNA と VEGF mRNA 医薬を用いた顎骨再生	課程博士 歯学		
G4305	江　傑	東莞市人民医院・副主任医師	日本医科大学大学院医学研究科 解析人体病理学	清水　章 教授	p. 188
		腎疾患の進展機序の解明とその制御	論文博士 医学		
G4306	王　晴	中国医科大学附属第四医院・主治医師	順天堂大学医学部 消化器外科講座上部消化管外科学	峯　真司 教授	p. 194
		食道癌に対する基礎的臨床的研究	論文博士 医学		
G4307	張　瑛	寧波市医療センター李恵利医院・主治医師	横浜市立大学大学院医学研究科 消化器内科学	前田　愼 主任教授	p. 210
		肝胆膵疾患・炎症性腸疾患における超音波を主体とした画像診断と治療	課程博士 医学		
G4308	葉　盛	南京紅十字血液センター・副主任医師	奈良県立医科大学大学院医学研究科 循環器システム医科学	中川　修 招聘教授 小亀 浩市 招聘教授	p. 226
		ADAMTS13 による VON WILLEBRAND 因子制御破綻がもたらす疾患の病態解析	課程博士 医学		
G4309	王　喻	京都大学大学院医学研究科・博士課程学生	京都大学大学院医学研究科附属 がん免疫総合研究センター	本庶　佑 センター長 京都大学高等研究院特別教授	p. 232
		PD-1 阻害による免疫賦活化異常疾患の研究	課程博士 医学		
G4310	孔 徳川	上海市疾病予防コントロールセンター・主治医師	熊本大学大学院医学教育部 ヒトレトロウイルス学共同研究センター 感染免疫学分野 国立感染症研究所感染病理部	上野 貴将 教授 徳永 研三 主任研究官	p. 238
		新型コロナウイルスの複製を制御する宿主因子の同定と機能解析	課程博士 医学		

日中笹川医学奨学金制度＜学位取得コース＞評価書
課程博士：指導教官用

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第 43 期　　研究者番号：G4301　　作成日　：　2024 年 3 月　11 日

氏　名	範 彬	FAN BIN	性別	M	生年月日　1987/09/01
所属機関（役職）	貴州医科大学附属医院病理科(住院医師)				
研究先（指導教官）	北海道大学大学院医学研究院病理学分野腫瘍病理学教室（田中 伸哉　教授）				
研究テーマ	**基質荷電を用いたドパミン作動性神経細胞への新規分化法の確立** Establishment of a novel method of differentiation into dopaminergic neurons using substrate charge				
専攻種別	□論文博士		☑課程博士		

研究者評価（指導教官記入欄）

成績状況	優　良　可　不可 学業成績係数=	取得単位数 取得単位数／取得すべき単位総数 19/16
学生本人が行った研究の概要	（研究概要）本研究では荷電ハイドロゲルを用いて human iPS 細胞の分化と基質荷電との関連について解析する。まず分化を試みる細胞はパーキンソン病の治療細胞としても注目されているドパミン作動性細胞である。ドパミン作動性細胞への分化誘導は多数報告があるが、いずれも高価な試薬を多くの試薬を組みあわせた複雑なプロトコールを有する。荷電との関連は報告がない。 （方法）本研究では以下の点について解析する。①human iPS 細胞のドパミン作動性細胞分化と基質荷電との関連について解析する。②基質荷電を用いた新たな分化プロトコールを確立する。③トランスクリプトーム解析、モデル動物への移植を行い、従来法で作成したドパミン作動性細胞との比較を行う。 （結果）２０２２年度では Human iPS 細胞を用いて、荷電ハイドロゲルにて分化誘導２８日目は、ドパミン作動性細胞マーカー（TH、CORIN、DAT、AADC）は高発現している結果を得た。ドパミン作動性細胞誘導することが出来た。基質荷電を用いた新たな分化プロトコールを確立した。２０２３年度では Human iPS 細胞を用いて、ドパミン誘導する酵素である AADC Knock out 細胞の樹立を行った。AADC Knock out 細胞は上流因子 L-DOPA の増加が見られた。 （今後の展望） 今年度はトランスクリプトーム解析を行い、さらにモデル動物への移植を行い、AADC Knock out 細胞により細胞療法を試み予定です。	
総合評価	【良かった点】 本人の取り込みが早く、現時点研究は順調に進んでおり、iPS 細胞の培養、iPS 細胞の荷電ハイドロゲルの誘導分化、RT-PCR の解析、ノックダウン細胞の樹立も完了致しました。 さらに今年度はトランスクリプトーム解析、モデル動物への移植を行い、細胞療法を試み予定です。大学院４年目で、論文の作成も取り込み中です。 本研究では世界初、荷電ハイドロゲルにより安価でドパミン作動性細胞の誘導が出来た。AADC Knock out 細胞により細胞療法は上手く行けば、今後臨床応用に大きく期待出来る。 【改善すべき点】 特になし。 【今後の展望】 まず、本人は大学院４年目で、論文のまとめ、学位の取得段階に入ります。 本研究おいては、今年度はトランスクリプトーム解析を行い、さらにモデル動物への移植を行い、AADC Knock out 細胞により細胞療法を試み予定です。細胞療法について、様々条件で検討しなければならないですが、上手く行けばパーキンソン病の臨床応用に大きく期待出来る。今年度は大学院４年目で、論文のまとめ、学位の取得段階に入ります。	
学位取得見込	学位取得見込み	

評価者（指導教官名）　　谷川　聖（田中　伸哉）

日中笹川医学奨学金制度<学位取得コース>報告書
研究者用

第43期　　研究者番号：G4301　　作成日：2024年3月 11 日

氏名	范 彬	FAN BIN	性別	M	生年月日 1987/09/01
所属機関(役職)	貴州医科大学附属医院病理科(住院医師)				
研究先（指導教官）	北海道大学大学院医学研究院病理学分野腫瘍病理学教室（田中 伸哉 教授）				
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専攻種別	論文博士	☐	課程博士	☑	

1．研究概要(1)

1)目的(Goal)To develop the protocol based on using an electrically charged hydrogel so that allowed to generate very consistent numbers of matured dopaminergic neurons from human iPSC lines which could generate L-DOPA.

2)戦略(Approach)

1.Induction of dopaminergic neuron differentiation from iPSCs using charged hydrogels

2.Development of AADC KO Dopaminergic neuron

3.Evaluation of function to synthesize L-DOPA

4.Animal model of Parkinson's disease (therapeutic experiment)

3)材料と方法(Materials and methods)

Materials:Induced pluripotent stem cells, iPSCs;Charged hydrogels;3-acrylamidopropyl-trimethyl-ammonium Chloride;2-Acrylamido-2-methylpropanesulfonic acid;In my research, I used 2 kinds of gels, C1A9 and C2A8, the C means positive charge while the A means negative charge. C1A9 means the kind of gel has 10% positive charge, and 90% negative charge.

Methods:1.Dopaminergic (DA) neurons differentiation culture on PS Dish or Charged hydrogels;2.Morphological observation on PS Dish or Charged hydrogels;3.Expression analysis of differentiation marker mRNA by real-time PCR;4.Protein expression analysis by Western blotting;5.Establishment of AADC knockout cell line using CRISPR/Cas9 system;6.Evaluation of L-DOPA and Dopamine production using HPLC and ELISA methods;7.Animal model of Parkinson's disease (therapeutic experiment)

4)実験結果(Results)

1.Using a protocol that induces dopaminergic neurons, induction with PS Dish was successful.

2.Moreover, using hydrogels, dopaminergic neurons were induced more efficiently than PS Dish.

3.AADC knockdown dopaminergic neurons were successfully induced on PS Dish.

4.Using hydrogel to simulate the neural microenvironment and efficiently induce dopaminergic neurons is expected to contribute to the development of translational medical technology for the treatment of refractory neurological diseases.

5)考察(Discussion)

Parkinson's disease is one of the most common neurological disorders. It is characterized by the loss of dopaminergic neurons. And the deficiency of L-DOPA is the main character of Parkinson's disease.

It is known that human pluripotent stem cells (PSCs) was successfully differentiated into DA neurons by Daisuke Doi[1] and the DA neurons had a good survival and lack of neural overgrowth indicate that it is promise for the development of cell-based therapies in Parkinson's disease.

However, it was reported [2] that the protocol has several problems: Low transfection efficiency, the DA neuron are not matured enough and the function of DA neurons largely depending on the source of hiPSCs.

It is known that the surface charge and wettability of artificial substrate are contributed to cell adhesion on scaffold, and surface charge of the substrates may directly affect adhesion of cellular membrane proteins.[3] [4]

Charged hydrogels electrically charged porous hydrogels can serve as scaffolds for brain parenchymal defects, and stepwise transplantation of NSCs into the hydrogel following gel implantation may induce the reconstruction of brain tissue along with the implanted hydrogels. Currently, various biomaterials have been used in human regenerative medicine, and biomedical engineering for specific tissues has become an important method to compensate for organ dysfunction.[5]

The available protocol of DA differentiation could be developed based on using an electrically charged hydrogel so that allowed to generate very consistent numbers of matured dopaminergic neurons from human iPSC lines.

And it was reported that Aromatic L-amino acid decarboxylase (AADC) could help L-DOPA change into dopamine, so if AADC is knocked out, the protocol may suspend in the step which L-DOPA change into dopamine, so the AADC-deficient dopaminergic cells may produce L-DOPA constantly.

Now, in the result of my research, it was proved the charged hydrogel could successfully induce dopaminergic neurons. Moreover, using hydrogels, dopaminergic neurons were induced more efficiently than PS Dish.

In the morphology of cells, the DA neurons appeared in Day 14, which was earlier than PS dish group. But as the charge of the hydrogel, the amount of cell is less than the PS dish group.

On the other hand, in the result of qPCR, the target genes TH, FOXA2, DAT, NURR and Lmx1B of hydrogel group were much higher than the control group. And in Western blot, the related protein was also higher than the control group, that means the charged hydrogel could actually develop the efficiently than PS Dish. The function of DA neurons was better than PS Dish. Especially the TH and DAT, was almost two folds to the PS Dish, meaning it could generate much more L-DOPA and Dopamine than in the PS dish.

Aromatic L-amino acid decarboxylase (AADC) could help L-DOPA change into dopamine. It could promote the combination of L-DOPA and TH to generate dopamine. AADC deficient attribute to the deficient synthesis of dopamine. So, the AADC-deficient dopaminergic cells may produce L-DOPA constantly.[6][7]

In this part, I am still working on it. First, it must be proven whether there is any difference between AADC knock out group and control group on PS dish. Then use the charged hydrogel to induce these two kinds of cells differentiating into DA neurons. Next, check whether there is any difference in L-DOPA and Dopamine between all the groups. Further research is required in the future..

6)参考文献(References)

[1] Daisuke Doi et al, Isolation of human induced pluripotent stem cell-derived dopaminergic progenitors by cell sorting for successful transplantation. Stem Cell Reports. 2014 Mar 6;2(3):337-50.
[2] Sameehan Mahajani et al, Homogenous generation of dopaminergic neurons from multiple hiPSC lines by transient expression of transcription factors. Cell Death Dis. 2019 Nov 27;10(12):898.
[3] Yusuke Arima te al, Effect of wettability and surface functional groups on protein adsorption and cell adhesion using well-defined mixed self-assembled monolayers. Biomaterials 28, 3074-3082 (2007).
[4] J H Lee et al, Cell behavior on polymer surfaces with different functional groups. Biomaterials. 1994 Jul;15(9):705-11.
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[6] Toni S. Pearson et al, AADC deficiency from infancy to adulthood: Symptoms and developmental outcome in an international cohort of 63 patients. J Inherit Metab Dis. 2020; 43:1121-1130.
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[1] Daisuke Doi et al, Isolation of human induced pluripotent stem cell-derived dopaminergic progenitors by cell sorting for successful transplantation. Stem Cell Reports. 2014 Mar 6;2(3):337-50.
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[7] Wassenberg et al. Consensus guideline for the diagnosis and treatment of aromatic l-amino acid decarboxylase (AADC) deficiency. Orphanet Journal of Rare Diseases (2017) 12:12.

2. 執筆論文 Publication of thesis ※記載した論文を添付してください。Attach all of the papers listed below.

論文名 1 Title						
掲載誌名 Published journal						
	年 月	巻(号)	頁 ～ 頁	言語 Language		
第1著者名 First author		第2著者名 Second author		第3著者名 Third author		
その他著者名 Other authors						
論文名 2 Title						
掲載誌名 Published journal						
	年 月	巻(号)	頁 ～ 頁	言語 Language		
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その他著者名 Other authors						
論文名 3 Title						
掲載誌名 Published journal						
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その他著者名 Other authors						
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その他著者名 Other authors						

3. 学会発表　Conference presentation　※筆頭演者として総会・国際学会を含む主な学会で発表したものを記載してくだ

※Describe your presentation as the principal presenter in major academic meetings including general meetings or international me

学会名 Conference									
演　題 Topic									
開催日 date	年	月	日	開催地 venue					
形式　method	□　口頭発表　Oral	□　ポスター発表　Poster		言語　Language	□　日本語	□　英語	□　中国語		
共同演者名 Co-presenter									
学会名 Conference									
演　題 Topic									
開催日 date	年	月	日	開催地 venue					
形式　method	□　口頭発表　Oral	□　ポスター発表　Poster		言語　Language	□　日本語	□　英語	□　中国語		
共同演者名 Co-presenter									
学会名 Conference									
演　題 Topic									
開催日 date	年	月	日	開催地 venue					
形式　method	□　口頭発表　Oral	□　ポスター発表　Poster		言語　Language	□　日本語	□　英語	□　中国語		
共同演者名 Co-presenter									
学会名 Conference									
演　題 Topic									
開催日 date	年	月	日	開催地 venue					
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共同演者名 Co-presenter									

4. 受賞(研究業績) Award (Research achievement)

名　称 Award name						
	国名 Country		受賞年 Year of		年	月
名　称 Award name						
	国名 Country		受賞年 Year of		年	月

5. 本研究テーマに関わる他の研究助成金受給 Other research grants concerned with your resarch them

受給実績 Receipt record	□ 有　□ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported period	年　月 ～　年　月
受給額 Amount received	円
受給実績 Receipt record	□ 有　□ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported period	年　月 ～　年　月
受給額 Amount received	円

6. 他の奨学金受給 Another awarded scholarship

受給実績 Receipt record	□ 有　□ 無
助成機関名称 Funding agency	
奨学金名称 Scholarship name	
受給期間 Supported period	年　月 ～　年　月
受給額 Amount received	円

7. 研究活動に関する報道発表 Press release concerned with your research activities

※記載した記事を添付してください。Attach a copy of the article described below

報道発表 Press release	□ 有　□ 無	発表年月日 Date of release	
発表機関 Released medium			
発表形式 Release method	・新聞　・雑誌　・Web site　・記者発表　・その他(　　)		
発表タイトル Released title			

8. 本研究テーマに関する特許出願予定 Patent application concerned with your research theme

出願予定 Scheduled	□ 有　□ 無	出願国 Application	
出願内容(概要) Application contents			

9. その他 Others

指導責任者(記名)　田中 伸哉

日中笹川医学奨学金制度＜学位取得コース＞評価書
課程博士：指導教官用

Supported by 日本財団 THE NIPPON FOUNDATION

第 43 期　　研究者番号：G4302　　作成日　：　2024 年 3 月 1 日

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研究テーマ	**アミノ酸トランスポーターを介した前立腺癌分子機構の解明（前列腺癌とアミノ酸トランスポーター）** Elucidation of the molecular mechanism of prostate cancer via amino acid transporter (prostate cancer and amino acid transporter)				
専攻種別	□論文博士		☑課程博士		

研究者評価（指導教官記入欄）

成績状況	㊧　良　可　不可 学業成績係数=4.0	取得単位数 45／30
学生本人が行った研究の概要	中間報告後の一年の研究活動の中で、前立腺癌とアミノ酸トランスポーターに関する総括論文を第一著者として、他に 5 つの論文を共同著者として発表した。第 33 回日本尿路結石症学会で口頭発表を行った。国立がん研究センターの特任研究員として、人工知能技術を用いてロボット支援前立腺癌手術のスキルを評価システムの開発に携わり、すでに成果を上げている。順調に研究を進め、博士論文を執筆している。	
総合評価	【良かった点】 詳細な専門知識と優れた学修能力を有している。研究プロジェクトでは、深い理解力と鋭い分析能力を発揮しており、学術分野での成功に必要な基盤を築いた。継続して学修する能力や課題解決能力に優れている。新規に着手した人工知能の研究領域や手法を積極的に探求し、難題に挑戦するとともに、冷静で粘り強い姿勢をもってそれらを克服し解決している。これらの探求心と課題解決能力は、将来の研究活動や職務における多大なる成果につながるものと評価している。また、人格も優れており、コミュニケーション能力の高さや他人に対して常に心を開き、同僚との良好なチームワークを築きながら研究を推進している。他人の意見も謙虚に聞き入れ、それを自分自身の行動に反映することにより、自分自身だけでなく研究グループとしての共通の目標の達成にも貢献している。当教室において無くてはならない人材として期待するとともに、高く評価している。 【改善すべき点】 中間評価で提示された点はすべて改善されている。 【今後の展望】 将来の展望に関して大きなポテンシャルを持ち、学術分野で優れた業績を上げる能力を持っていると確信している。継続的な努力と学修、研究の深化、そして積極的な学術交流と協力により、当該領域に新しい思考や成果をもたらし、その分野を切り開く先駆者となることを期待している。	
学位取得見込	十分な学術的基盤と研究の潜在能力を持っている。専心して学び、努力し、自身の優位性を最大限に発揮すれば、博士号を取得するだけでなく、研究領域において多大なる成果を挙げることができると確信している。	
評価者（市川　智彦）		

日中笹川医学奨学金制度<学位取得コース>報告書
研究者用

第43期　　研究者番号：　G4302　　作成日：2024年3月1日

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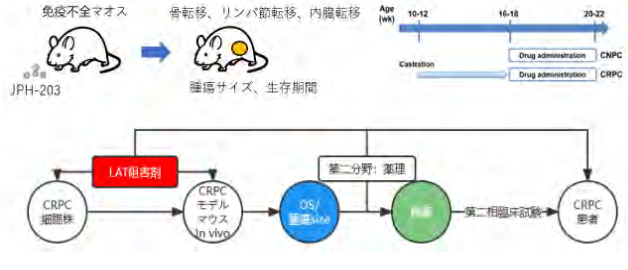
研究先（指導教官）	千葉大学大学院 医学研究院泌尿器科学（市川 智彦　教授）				
研究テーマ	アミノ酸トランスポーターを介した前立腺癌分子機構の解明（前列腺癌とアミノ酸トランスポーター） Elucidation of the molecular mechanism of prostate cancer via amino acid transporter (prostate cancer and amino acid transporter)				
専攻種別	論文博士	☐	課程博士	☑	

1．研究概要（1）

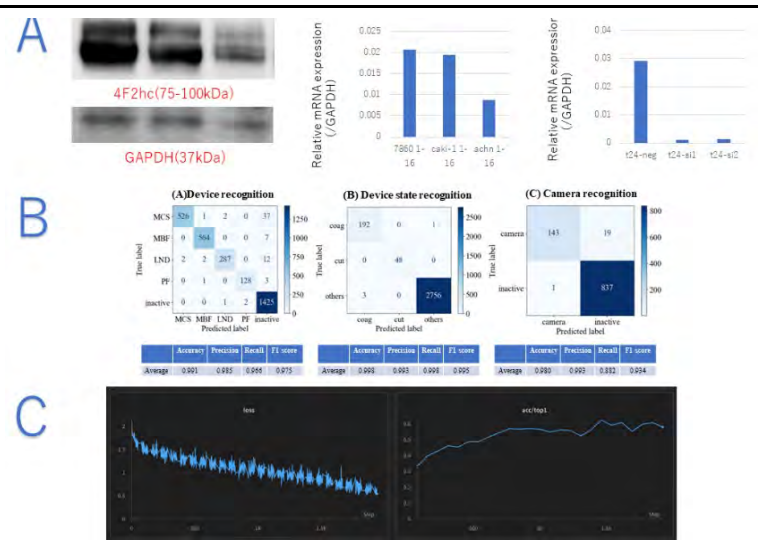
1）目的（Goal）
目的：①アミノ酸トランスポーター阻害剤の去勢抵抗性前立腺癌（CRPC）に対する作用効果の解明（In vivo）。②LAT1を含めたトランスポーターを介したCRPC治療モデルの構築と、実臨床への応用を目指す。③前立腺がんに関する人工知能技術を用いた臨床研究が行われている。
研究背景：社会の高齢化が進むにつれて、前立腺癌の発病率は年々増加している。前立腺癌の治療において、無視できないのは、前立腺癌が最終的に去勢抵抗型前立腺癌（CRPC）に転換することである。我々は、前立腺癌がCRPCに移行するメカニズムとして、アンドロゲン受容体（AR）に制御されるトランスポーターとしてLAT1（LAT1-4F2hcヘテロダイマー型トランスポーター）を同定した[1]。LAT1複合体は前立腺癌において特異的な発現亢進が報告されている[2]。さらに、SCL3A2遺伝子（4F2hc）はCRPCに関与するアンドロゲン受容体のスプライスバリアント（AR-V7）の特異的標的遺伝子であることを発見した[1]。LAT1阻害剤（JPH 203）はすでに消化器腫瘍において第一相臨床試験を完了し、良好な結果を得た[3]。SGLT-2阻害剤は糖尿病で臨床応用されていることから、LAT1を含めたアミノ酸トランスポーターの阻害剤もCRPC患者において臨床応用の可能性が示唆される。本研究の目的はLAT1を含むアミノ酸トランスポーターの阻害剤を応用した、去勢抵抗性前立腺癌（CRPC）治療モデル（In vivio）の構築を提案する。最終的には、第二相臨床試験をCRPC患者において実現させる。近年、人工知能技術が飛躍的に進歩し、臨床分野での応用例や研究の方向性が出てきていることから、人工知能による泌尿器科の研究をテーマにしている。
2）戦略（Approach）
千葉大学泌尿器科学研究室は、2016年からアミノ酸トランスポーターの第一人者である大阪大学　金井好克教授、千葉大学　安西尚彦教授、LAT1阻害剤を供給するジェイファーマ株式会社らと共同研究を行っている。複数の先行研究成果[1,2,4-7]と豊富な共同研究経験があり、後続研究の実現が可能である。 研究者本人はLAT1-4F2hc複合体と前立腺癌に関する総説論文2編が発表され、前立腺癌とLAT 1複合体の分野に対して比較的に十分な理解と認識を持っている。
　LAT阻害剤（JPH203）の利用：大阪大学 金井好克教授、ジェイファーマ株式会社とのMTAを締結済。共同研究として、LAT1の特異的阻害剤（JPH203）の臨床応用へ向けた解析を進める。すでに、膵臓癌と胆管癌にて第一層臨床試験UMIN000016840終了しており、軽度の副作用（12%のAST上昇）と25カ月の長期奏功例を胆管癌患者に認めている。
　本研究では、上記の先行研究と共同プロジェクトに基づいて、3つのステップで結論を導き出すことが期待される。現在の展望は以下の通り記述する。①前立腺がんCRPC細胞株の確立、LAT1関系の検証です。②CRPCモデルマウスの作成、JPH203を用いた治療実験を行う。③上記の①②の調査で得られたデータをもとに比較分析して臨床利用の妥当性を判断する。3つのステップを3年計画として、1年目は計画通りに前立腺がんとLAT1の関係を調べ、細胞の培養や実験を行う。調査をもとにまとめたレビュー論文2本が学術誌に掲載されました。2年目には細胞のスクリーニングに成功し、siRNAを使って4F2hc遺伝子をノックアウトしました。ノックダウン後の細胞の増殖、侵襲、転移の様子が観察されています。また国立がん研究センターの特任研究員として、ロボットで支援する前立腺がん手術（RARP）をai分析評価システムの開発にも携わりました。3年目は主にマウスモデルの作成と博士論文の作成を行う。
3）材料と方法（Materials and methods）
研究方法及び内容：免疫不全マウスの皮下移植前立腺癌モデルを用いてLAT1阻害剤（JPH203）臨床応用へ向けたIn Vivo解析を行う。
① CRPC細胞系（LNCaP、DU145、PC-3）におけるLAT阻害剤の作用効果の解明。（細胞形態、成長、分化、代謝、アポトーシス、信号伝導経路など。）
・CRPC細胞系（LNCaP、DU145、PC-3）のLAT1-4F2hcをrtPCRで検証、LAT1-4F2hcのタンパク質量をWestern-Blottingで検証。
・siRNAを作用させるCRPC細胞系のLAT1-4F2hc量と関連タンパク質量をrtPCRとWestern-Blottingで検証。
・2種類の細胞株を選択し、JPH203を投入する。LAT1-4F2hc量と関連タンパク質量をrtPCRとWestern-Blottingで検証。
・JPH203投入後の細胞株で細胞増殖実験、migration/invasion assayなど、細胞の成長、侵襲、転移などの能力を比較する。
② 去勢、非去勢モデルにて16w-18wにて薬剤投与（経静脈的）を開始し、腫瘍増大抑制効果とマウスの生存期間延長効果を解析する。
・前立腺腫瘍細胞を6 ~ 8週目の免疫不全のオスのヌードマウスをランダムに4つのグループ(去勢/非去勢治療群と対照群)に分けて皮下注射し、細胞を入れてから24時間後にバイオライトイメージングで腫瘍の成長を監視し、その後2週間ごとに4週間監視する。治療群は16 ~ 18周間にJPH203を使用し、バイオライトイメージングで
腫瘍の変化を監視し、画像ソフトウェアで定量的な比較する。
グループ間の生存期間を比較する。
③ 得られた結果と抗腫瘍効果、副作用を含めて分析し、
臨床応用の可能性を探索する。



1．研究概要（2）
4）実験結果（Results）
A. 4F2hc高発現の2種類の細胞を選別し、westernとrtPCRで検証し、siRNAを用いて4F2hcをノックダウンしました。ノックダウン後の細胞の増殖、侵襲、転移の様子が観察されています。

国立がん研究センターの特任研究員として、ロボットで支援する前立腺がん手術（RARP）をai分析評価システムの開発にも携わりました。
B.画像認識技術に基づいたRARP手術ユーザーインターフェース認識モデルを構築し、手術器具とCUT/COAGオン操作の認識精度を高めました(0.98~0.998)

C.現在、学習中の人工知能がRARP手術縫合の操作を認識するモデルを構築中で、精度は60%に達しています(学習中)。



5）考察（Discussion）
5.1）抗アンドロゲン治療(ADT)におけるLAT1の上昇が、前立腺がん細胞の進行を促すことがわかっている[2]。LAT1はCRPC細胞株で強く発現する。LAT1ノックアウトは細胞の増殖、移動、侵襲を著しく減少させる。慢性ADTの患者では、LAT1の高発現は生化学的無再発期の低下と相関している[2]。慢性ADTを伴う22Rv1 CRPC腫瘍において、LAT1のタンパク質およびmRNAレベルでの発現が増加していることが確認されている[8]。SugiuraはAR-v7とLAT1-4F2hc複合体の潜在的な関係を示しました。AR-v7はアンドロゲンの欠乏で下流の標的遺伝子を活性化する。4F2hcはAR-v7の下流のターゲットの1つです。CRPC組織における4F2hc発現レベルの有意な上昇は、予後不良を示唆している[9]。
トランスポーターの阻害剤には、輸送化合物と、非輸送化合物がある。現在の薬理学では、細胞内に蓄積せず、親和性が高い非輸送系化合物が輸送系化合物より優れていると考えられている[10]。JPH203 (KYT-0353)は、2009年にLAT1特異的阻害剤として開発されました[11]。そして、JPH203は最近、LAT1の効果的な阻害剤として広く研究されている。JPH203は、mTORC1とAktの組成活性化を妨害し、c-Mycの発現を低下させ、細胞死に関与するCHOP転写因子が介在するフラックタンパク反応を誘発する[12]。第I相臨床研究では、JPH203には良好な耐性があり、胆道癌治療の予後が良好であることが報告されており、胆道癌に対する疾患抑制率は約60%でした[3]。ですから、我々はCRPCでJPH203の第I相と第II相の研究を行う予定です。また、日本の研究チームはT3やJPH203に似たSKN系LAT1阻害剤[13,14]を開発しました。近年、大阪大学の金井教授らの研究チームは、新しいLAT1阻害剤「OKYシリーズ」を開発している。OKY化合物では、OKY-034はLAT1に対して高い阻害性と特異性を示しました。上記のアミノ酸LAT1阻害剤は競合阻害剤ですが、OKY-034はアミノ酸の骨格を持たないため、非競合阻害剤のスタイルを持っています。非競合阻害剤は、内因性アミノ酸基質と競合的に反応する必要があるため、少量(低濃度)で効果を示すことができます。また、OKY-034はT3やSKNといった大きな疎水点を必要としないため、比較的水溶性に優れ、経口投与が可能です。膵臓がん患者におけるOKY-034の安全性と有効性のI/IIa相試験は大阪大学病院で行われている(UMIN000036395)[15]。これらの薬はまもなく前立腺がんの治療に使われる。
前立腺がんにおけるアミノ酸トランスポーターLAT1-4F2hc複合体の臨床的意義は、他の腫瘍細胞と同様に、次第に解明されつつある。LAT1-4F2hcは前立腺がんの診断、治療、予後評価に重要な役割を果たしている。前立腺がんに関連するアミノ酸トランスポーター阻害剤であるJPH203は、近い将来泌尿器系腫瘍の診断と治療戦略を変える可能性がある。

5.2）23年以上も臨床に応用されてきたロボット補助根治前立腺切除術(RARP)[16]は患者数は年々増加している。現在、米国の根治前立腺切除術の約70%がこの方法で行われています[17]。医者の技術と予後には有意な関係があり、患者の予後に影響を与える重要な要因の1つです[18-20]。しかし、現在の主流の外科技術評価システムは、専門家評価表モデル[21]に基づいています。例えば、腹腔鏡手術スキルの総合評価(GOALS)[22]、技術スキルの客観的構造化評価(OSATS)[23]、医療失敗パターンと効果分析(HFMEA)[24]などです。これらの評価ツールは、評価に時間がかかるだけでなく、専門家の労力と個人的な判断を必要とします。評価の専門家が介在することで、評価結果が主観的になることは避けられません。そのため、手術のスキル評価をいかに自動化、効率化し、客観的な評価結果にするかが重要な課題となっています。私たちの人工知能(AI)による画像認識技術モデルが、この問いに答える方向を示しているようです。

6）参考文献（References）
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2. 執筆論文 Publication of thesis ※記載した論文を添付してください。Attach all of the papers listed below.

論文名 1 Title	Tumor Location and a Tumor Volume over 2.8 cc Predict the Prognosis for Japanese Localized Prostate Cancer.					
掲載誌名 Published journal	Cancers					
	2022 年 12 月	14 (23) 巻(号)	5823 頁 ～ 5836 頁	言語 Language	英語	
第1著者名 First author	Xue Zhao	第2著者名 Second author	Shinichi Sakamoto	第3著者名 Third author	Haruki Baba	
その他著者名 Other authors	Yasutaka Yamada, Junryo Rii, Ayumi Fujimoto, Manato Kanesaka, Nobuyoshi Takeuchi, Tomokazu Sazuka, Yusuke Imamura, Koichiro Akakura, Tomohiko Ichikawa.					
論文名 2 Title	Targeting L-type amino acid transporter 1 in urological malignancy: Current status and future perspective.					
掲載誌名 Published journal	J Pharmacol Sci					
	2022 年 12 月	150 (4) 巻(号)	251 頁 ～ 258 頁	言語 Language	英語	
第1著者名 First author	Sangjon Pae	第2著者名 Second author	Shinichi Sakamoto	第3著者名 Third author	Xue Zhao	
その他著者名 Other authors	Shinpei Saito, Takaaki Tamura, Yusuke Imamura, Tomokazu Sazuka, Yoshie Reien, Yuri Hirayama, Hirofumi Hashimoto, Yoshikatsu Kanai, Tomohiko Ichikawa, Naohiko Anzai.					
論文名 3 Title	Contribution of LAT1-4F2hc in Urological Cancers via Toll-like Receptor and Other Vital Pathways.					
掲載誌名 Published journal	Cancers					
	2022 年 1 月	14 (1) 巻(号)	229 頁 ～ 248 頁	言語 Language	英語	
第1著者名 First author	Xue Zhao	第2著者名 Second author	Shinichi Sakamoto	第3著者名 Third author	Maihulan Maimaiti	
その他著者名 Other authors	Naohiko Anzai, Tomohiko Ichikawa.					
論文名 4 Title	Serum Testosterone Level Determines the Treatment Strategy of Advanced Prostate Cancer.					
掲載誌名 Published journal	Horizons in Cancer Research (Book)					
	2023 年 4 月	85 巻(号)	Chapter 3 頁 ～ Chapter 3 頁	言語 Language	英語	
第1著者名 First author	Xue Zhao	第2著者名 Second author	Shinichi Sakamoto	第3著者名 Third author	Shuhei Kamada	
その他著者名 Other authors	Akinori Takei, Yusuke Imamura, Tomohiko Ichikawa.					
論文名 5 Title	Contribution of the L-Type Amino Acid Transporter Family in the Diagnosis and Treatment of Prostate Cancer.					
掲載誌名 Published journal	International Journal of Molecular Sciences					
	2023 年 2 月	24 巻(号)	6178 頁 ～ 6195 頁	言語 Language	英語	
第1著者名 First author	Xue Zhao	第2著者名 Second author	Shinichi Sakamoto	第3著者名 Third author	Jiaxing Wei	
その他著者名 Other authors	Sangjon Pae, Shinpei Saito, Tomokazu Sazuka, Yusuke Imamura, Naohiko Anzai, Tomohiko Ichikawa.					

2. 執筆論文 Publication of thesis ※記載した論文を添付してください。Attach all of the papers listed below.

論文名 6 Title	Machine- learning predicts time-series prognosis factors in metastatic prostate cancer patients treated with androgen deprivation therapy.				
掲載誌名 Published journal	Scientific Reports				
	2023 年 4 月	13(1) 巻(号)	6325 頁 ～ 6334 頁	言語 Language	英語
第1著者名 First author	Shinpei Saito	第2著者名 Second author	Shinichi Sakamoto	第3著者名 Third author	Kosuke Higuchi
その他著者名 Other authors	Kodai Sato, Xue Zhao, Ken Wakai, Manato Kanesaka, Shuhei Kamada, Nobuyoshi Takeuchi, Tomokazu Sazuka, Yusuke Imamura, Naohiko Anzai, Tomohiko Ichikawa, Eiryo Kawakami.				
論文名 7 Title	The Oncological and Functional Prognostic Value of Unconventional Histology of Prostate Cancer in Localized Disease Treated with Robotic Radical Prostatectomy: An International Multicenter 5-Year Cohort Study				
掲載誌名 Published journal	EUROPEAN UROLOGY ONCOLOGY				
	2024 年 1 月	23 巻(号)	印刷中 頁 ～ 頁	言語 Language	英語
第1著者名 First author	David Leung	第2著者名 Second author	Daniele Castellani	第3著者名 Third author	Rossella Nicoletti
その他著者名 Other authors	Roser Vives Dilme, Jesus Moreno Sierra, Sergio Serni, Carmine Franzese, Giuseppe Chiacchio, Andrea Benedetto Galosi, Roberta Mazzucchelli, Erika Palagonia, Paolo Dell'Oglio, Antonio Galfano, Aldo Massimo Bocciardi, Xue Zhao, Chi Fai Ng, Hsiang Ying Lee, Shinichi Sakamoto, Nikhil Vasdev, Juan Gomez Rivas, Riccardo Campi, Jeremy Yuen-Chun Teoh.				
論文名 8 Title	Tumor localization by Prostate Imaging and Reporting and Data System (PI-RADS) version 2.1 predicts prognosis of prostate cancer after radical prostatectomy.				
掲載誌名 Published journal	Scientific Reports				
	2023 年 6 月	13(1) 巻(号)	10079 頁 ～ 10088 頁	言語 Language	英語
第1著者名 First author	Ayumi Fujimoto	第2著者名 Second author	Shinichi Sakamoto	第3著者名 Third author	Takuro Horikoshi
その他著者名 Other authors	Xue Zhao, Yasutaka Yamada, Junryo Rii, Nobuyoshi Takeuchi, Yusuke Imamura, Tomokazu Sazuka, Keisuke Matsusaka, Jun-Ichiro Ikeda, Tomohiko Ichikawa.				
論文名 9 Title	Preoperative PI-RADS v2.1 Scoring System Improves Risk Classification in Patients Undergoing Radical Prostatectomy.				
掲載誌名 Published journal	Anticancer Research				
	2023 年 12 月	43(12) 巻(号)	5705 頁 ～ 5712 頁	言語 Language	英語
第1著者名 First author	Yudai Fukui	第2著者名 Second author	Yasutaka Yamada	第3著者名 Third author	Shinichi Sakamoto
その他著者名 Other authors	Takuro Horikoshi, Xue Zhao, Kodai Sato, Sakie Nanba, Yoshihiro Kubota, Manato Kanesaka, Ayumi Fujimoto, Hiroki Shibata, Yusuke Goto, Tomokazu Sazuka, Yusuke Imamura, Takashi Uno, Tomohiko Ichikawa.				
論文名 10 Title	Copy Number Gain in Androgen Receptors Predicts the Poor Prognosis in Japanese Castration-resistant Prostate Cancer.				
掲載誌名 Published journal	Anticancer Research				
	2024 年 2 月	44(2) 巻(号)	639 頁 ～ 647 頁	言語 Language	英語
第1著者名 First author	Shinichi Sakamoto	第2著者名 Second author	Keisuke Ando	第3著者名 Third author	Sangjon Pae
その他著者名 Other authors	Xue Zhao, Kazuko Sakai, Kodai Sato, Shinpei Saito, Yasutaka Yamada, Junryo Rii, Yusuke Goto, Tomokazu Sazuka, Yusuke Imamura, Naohiko Anzai, Koichiro Akakura, Kazuto Nishio, Tomohiko Ichikawa.				

3. 学会発表 Conference presentation ※筆頭演者として総会・国際学会を含む主な学会で発表したものを記載してくださ

※Describe your presentation as the principal presenter in major academic meetings including general meetings or international meetin

学会名 Conference	日本尿路結石症学会第33回学術集会						
演題 Topic	SWLハイボリュームセンターにおけるSkin to Stone Distance(SSD)と尿管結石破砕効率に関する検討						
開催日 date	2023 年 8 月 25 日		開催地 venue	久留米市			
形式 method	☑ 口頭発表 Oral	□ ポスター発表 Poster	言語 Language	☑ 日本語	□ 英語	□ 中国語	
共同演者名 Co-presenter	坂本 信一，野積 和義，柴田 裕貴，山田 康隆，五島 悠介，今村 有佑，市川 智彦						
学会名 Conference							
演題 Topic							
開催日 date	年 月 日		開催地 venue				
形式 method	□ 口頭発表 Oral	□ ポスター発表 Poster	言語 Language	□ 日本語	□ 英語	□ 中国語	
共同演者名 Co-presenter							
学会名 Conference							
演題 Topic							
開催日 date	年 月 日		開催地 venue				
形式 method	□ 口頭発表 Oral	□ ポスター発表 Poster	言語 Language	□ 日本語	□ 英語	□ 中国語	
共同演者名 Co-presenter							
学会名 Conference							
演題 Topic							
開催日 date	年 月 日		開催地 venue				
形式 method	□ 口頭発表 Oral	□ ポスター発表 Poster	言語 Language	□ 日本語	□ 英語	□ 中国語	
共同演者名 Co-presenter							

4. 受賞（研究業績） Award (Research achievement)

名称 Award name				
	国名 Country		受賞年 Year of award	年 月
名称 Award name				
	国名 Country		受賞年 Year of award	年 月

5. 本研究テーマに関わる他の研究助成金受給 Other research grants concerned with your resarch theme

受給実績 Receipt record	■ 有　□ 無
助成機関名称 Funding agency	国立研究開発法人 科学技術振興機構（JST）
助成金名称 Grant name	全方位イノベーション創発博士人材養成プロジェクト
受給期間 Supported period	2023 年 4 月 ～ 2024 年 3 月
受給額 Amount received	770,000 円
受給実績 Receipt record	□ 有　□ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円

6. 他の奨学金受給 Another awarded scholarship

受給実績 Receipt record	□ 有　■ 無
助成機関名称 Funding agency	
奨学金名称 Scholarship name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円

7. 研究活動に関する報道発表 Press release concerned with your research activities

※記載した記事を添付してください。Attach a copy of the article described below

報道発表 Press release	□ 有　■ 無	発表年月日 Date of release	
発表機関 Released medium			
発表形式 Release method	・新聞　・雑誌　・Web site　・記者発表　・その他（　　）		
発表タイトル Released title			

8. 本研究テーマに関する特許出願予定 Patent application concerned with your research theme

出願予定 Scheduled	□ 有　■ 無	出願国 Application	
出願内容（概要） Application contents			

9. その他 Others

指導責任者（記名） 市川 智彦

cancers

MDPI

Article

Tumor Location and a Tumor Volume over 2.8 cc Predict the Prognosis for Japanese Localized Prostate Cancer

Haruki Baba [1,†], Shinichi Sakamoto [1,*,†], Xue Zhao [1,†], Yasutaka Yamada [1], Junryo Rii [1], Ayumi Fujimoto [1], Manato Kanesaka [1], Nobuyoshi Takeuchi [1], Tomokazu Sazuka [1], Yusuke Imamura [1], Koichiro Akakura [2] and Tomohiko Ichikawa [1]

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† These authors contributed equally to this work.

Simple Summary: About 40% of men with localized prostate cancer experience biochemical recurrence after radical prostatectomy. The early detection of disease progression is important for optimal post-operative treatment and follow-up. Our study reviewed 557 patients with prostate cancer who underwent radical prostatectomy and found that a tumor volume over 2.8 cc was a novel independent predictive factor for biochemical recurrence. We further established a novel risk assessment model based on tumor volume and location (posterior and peripheral zone). We confirmed that the risk model could stratify patients' prognoses. In addition to the previously reported biomarkers, these novel factors obtained from the surgical specimen may provide better prognostic information in patients with prostate cancer.

Abstract: (1) Objective: Our study investigated the prognostic value of tumor volume and location in prostate cancer patients who received radical prostatectomy (RP). (2) Methods: The prognostic significance of tumor volume and location, together with other clinical factors, was studied using 557 patients who received RP. (3) Results: The receiver operating characteristic (ROC) curve identified the optimal cutoff value of tumor volume as 2.8 cc for predicting biochemical recurrence (BCR). Cox regression analysis revealed that a tumor in the posterior area (p = 0.031), peripheral zone (p = 0.0472), and tumor volume $\geq$ 2.8 cc (p < 0.0001) were predictive factors in univariate analysis. After multivariate analysis, tumor volume $\geq$ 2.8 cc (p = 0.0225) was an independent predictive factor for BCR. Among them, a novel risk model was established using tumor volume and location in the posterior area and peripheral zone. The progression-free survival (PFS) of patients who met the three criteria (unfavorable group) was significantly worse than other groups ($p \leq 0.001$). Furthermore, multivariate analysis showed that the unfavorable risk was an independent prognostic factor for BCR. The prognostic significance of our risk model was observed in low- to intermediate-risk patients, although it was not observed in high-risk patients. (4) Conclusion: Tumor volume (≥2.8 cc) and localization (posterior/peripheral zone) may be a novel prognostic factor in patients undergoing RP.

Keywords: tumor volume; tumor location; prostate cancer; biochemical recurrence; prognostic factor

Citation: Baba, H.; Sakamoto, S.; Zhao, X.; Yamada, Y.; Rii, J.; Fujimoto, A.; Kanesaka, M.; Takeuchi, N.; Sazuka, T.; Imamura, Y.; et al. Tumor Location and a Tumor Volume over 2.8 cc Predict the Prognosis for Japanese Localized Prostate Cancer. *Cancers* **2022**, *14*, 5823. https://doi.org/10.3390/cancers14235823

Academic Editor: Grace Lu-Yao

Received: 5 November 2022
Accepted: 24 November 2022
Published: 25 November 2022

1. Introduction

Prostate cancer (Pca) is the most common malignant tumor in men. About 2.6 million cases are newly diagnosed and 34,500 deaths of Pca are estimated per year in the United States [1]. Radical prostatectomy (RP) for the treatment of prostate cancer has made remarkable progress since it widely emerged around 1900. At present, RP is still the standard treatment option for localized Pca [2]. However, the frequency of biochemical recurrence (BCR) has been reported to be about 40% within 10 years after RP [3]. Once BCR

occurs, about 3.5% of patients will inevitably develop resistance to androgen deprivation therapy, also known as castration-resistant prostate cancer (CRPC) [4]. CRPC has been reported to cause death within 2 to 4 years [5]. Therefore, BCR is the major clinical issue to be detected and addressed in patients who received RP.

A lot of clinical studies have evaluated predictive factors and/or risk models for BCR after RP. Serum prostate-specific antigen (PSA) is the mainstay to detect the BCR of patients after surgery [6], and it has been recommended to keep close monitoring until PSA reaches 0.2 ng/mL [7]. In addition to PSA kinetics, Gleason score, PSA density, pathological and clinical stages, surgical margin, and other clinical factors have been studied for their prognostic significance, however, these factors could not predict BCR independently [8]. To better distinguish the recurrence risk and evaluate the prognosis after RP, more innovative predictors or models are unmet clinical needs. The individualized management after treatment requires effective recurrence risk prediction to implement timely intervention and avoid overtreatment. Previous studies showed that the tumor volume was related to the clinical manifestations of prostate cancer [9]. A tumor with a volume of less than 0.5 cc is considered as insignificant prostate cancer, and aggressive treatment may not be needed [10,11]. Recently, several studies proposed the novel definition of insignificant prostate cancer as a tumor volume of less than 2.5 cc [11–17], or less than 2.0 cc [18]. However, it was found that the BCR risk increased with tumor volume over 2.49 cc, indicating that the tumor volume was deeply involved in the progression of Pca [19]. Furthermore, little is known about the relationship between different prostate areas and tumor volumes, and their impact on BCR. Herein, we examined the prognostic role of tumor volume and location in patients with localized Pca for a better treatment strategy and postoperative follow-up.

2. Methods

2.1. Study Design and Setting

Clinical data of 557 patients who received RP at Chiba University Hospital and affiliated hospitals between 2006 and 2020 were retrospectively reviewed. The study was approved by the clinical review committee of our institution (#1768) and the written informed consent of all patients participating in the study was obtained. All participants or designated agents accepted a standardized data collection protocol, including personal postoperative follow-up information and medical record. The study is in accordance with the Japanese ethical document.

2.2. Patients

The inclusion criteria were RP for biopsy-proven prostate cancer performed at Chiba University Hospital and affiliated hospitals; whole-mount step-section pathologic maps available for tumor volume-calculation and localization. The exclusion criteria were neoadjuvant hormone therapy; radiation therapy; poor pathologic map quality; short follow-up term (<12 months).

2.3. Variables

Baseline clinical data included age, BMI, serum PSA, PSA F/T ratio, serum testosterone, biopsy positive rate, Gleason score (GS), clinical TNM staging, surgical prostate specimen, tumor volume, tumor location, surgical resect margin, and pathological TNM staging. Each patient came to our institution every 3 months after RP and had blood samples taken for PSA measurement until the occurrence of BCR or death was confirmed.

After RP, an elevated serum PSA level (>0.2 ng/mL) was defined as BCR [6].

2.4. Tumor Volume and Location Estimation Method

2.4.1. Measurement of Tumor Volume

The prostatectomy specimens were step-sectioned transversely at 5-mm intervals. All the specimens were mounted on slides. Tumor volume was calculated by scanning the

and had blood samples taken for PSA measurement until the occurrence of BCR or death was confirmed.

After RP, an elevated serum PSA level (>0.2 ng/mL) was defined as BCR [6].

2.4. Tumor Volume and Location Estimation Method

2.4.1. Measurement of Tumor Volume

The prostatectomy specimens were step-sectioned transversely at 5-mm intervals. All the specimens were mounted on slides. Tumor volume was calculated by scanning the sliced specimen, and the area of the tumor was analyzed using ImageJ software. Total tumor volume = tumor area × thickness of specimen × 1.2 (correction for shrinkage).

2.4.2. Tumor Localization

All specimens were serially sectioned from the tip to the base at 5 mm intervals, and the bladder neck and vertex edges were submitted as vertical sections. According to the anatomical structure, the specimen was divided into the following regions: the peripheral zone (PZ), the transition zone (TZ), and the central zone (CZ). The region within 1.0 cm or 1.5 cm from the tip of the prostate was identified as the Apex region. The prostatic urethra is an anatomic marker for a tumor to be classified as anterior or posterior (Figure 1). If a tumor showed a slight extension to another site, >80% volume in the main area was the criterion for defining the origin of the tumor in this area. Each RP sample was reviewed by two pathologists.

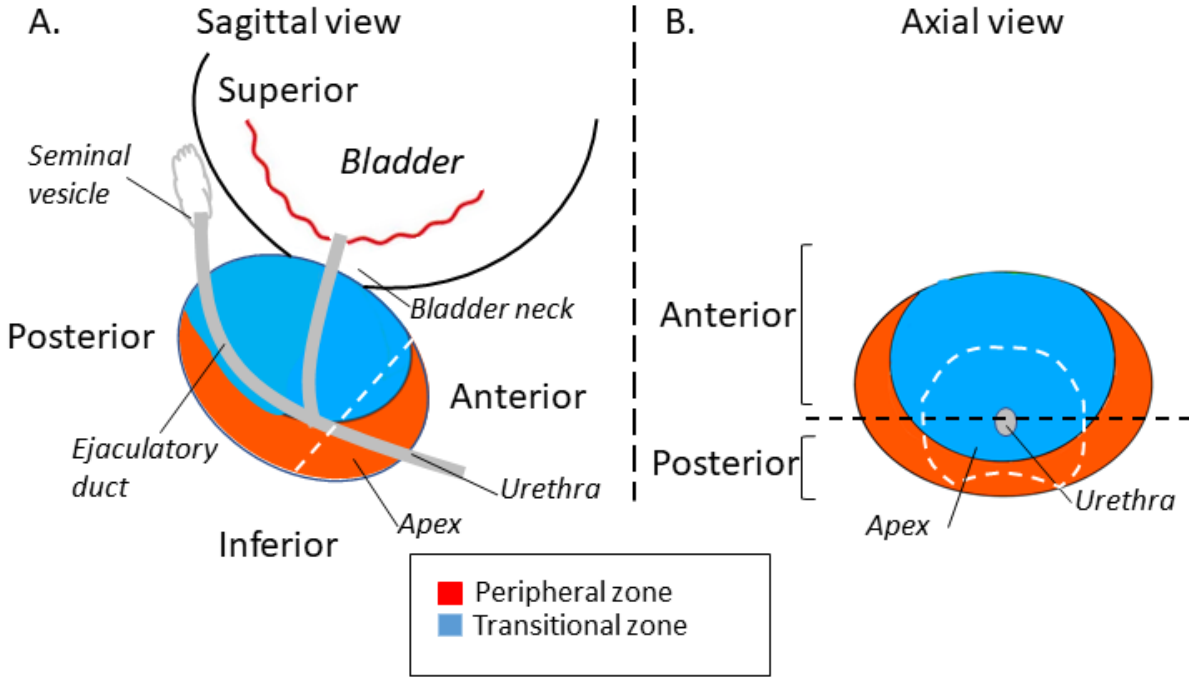

Figure 1. Schematic diagram of an anatomical division of the prostate. The location of the Anterior/Posterior and Peripheral/Transitional Zones are described. (**A**) Sagittal view. (**B**) Axial view.

2.5. Statistical Methods

JMP Pro (Version 16.0; SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. Univariate cox proportional hazards model analysis was performed on the baseline data classified by the median value of the outcome measurement to determine the predictive factors of the BCR. The significant variables ($p < 0.05$) were further analyzed by multivariable cox proportional hazards model regression. The optimal cutoff value of tumor volume was obtained by calculating Area Under the Curve (AUC) from the Receiver Operating Characteristic (ROC) curve analysis. To evaluate the interaction between tumor volume and location, 3 risk factors related to volume and location obtained from univariate and multivariate cox regression analysis were combined into a risk classification model. This model was grouped according to the number of risk factors displayed: favorable; 0 risk factor, moderate; 1 or 2 risk factors, unfavorable; all 3 risk factors. Kaplan–Meier method was used to evaluate progression-free survival (PFS). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Participants

In total, 557 patients were enrolled in the study. Follow-up terms ranged from 12 to 161.5 months, with a median follow-up time of 45.3 months. As of the end of the study, 66 (11.8%) patients had BCR, and 9 (1.6%) patients died (not due to prostate cancer). The median age of all patients was 67 years old. The median preoperative PSA level was 7.71 ng/mL. The biopsy GS was 7 or less in 79.7%, 8 in 8.6%, and 9 or more in 11%. Overall, 64.8% of patients were pathological TNM stage 2c or above, and 1.4% were positive for lymph node metastasis. According to the risk grouping of Pca by the American Cancer Society (ACS), 77 (13.8%) patients were classified into the low-risk group, 279 (50.1%) were classified into the intermediate-risk group, and 201 (36.1%) were classified into the high-risk group. The median tumor volume was 2.12 cc. Seminal vesicle invasion was observed in 8.6%, the extracapsular invasion was seen in 24.8%, and 30.3% had positive margins. The tumor distributions were in the apex area (63.7%), middle area (63.4%), and bladder neck (21.4%). Regarding the anterior or posterior area of the prostate, 48.1% of the tumors were in the anterior, and 52.4% were in the posterior. Overall, 67.1% were located in the PZ and 37.3% were in the TZ (Table 1).

Table 1. Characteristics of patients.

Characteristics	
Number of patients	557
Median age at operation (range), years	67 (46–77)
Median follow-up time (range), months	45.3 (12–161.5)
Median initial PSA (range) (ng/mL)	7.71 (2.15–87.16)
Gleason score sum, n (%)	
≤7	444 (79.7)
8	48 (8.6)
≥9	61 (11.0)
T stage, n (%)	
≤2b	195 (35.0)
≥2c	361 (64.8)
Risk Group; Low/Intermediate/High, n (%)	77 (13.8)/279 (50.1)/201 (36.1)
Tumor Volume (range), cc	2.12 (0.02–57)
Tumor Location, n (%)	
apex	355 (63.7)
middle	353 (63.4)
bladder neck	119 (21.4)
Tumor Location, n (%)	
anterior	268 (48.1)
posterior	292 (52.4)
Tumor Location, n (%)	
PZ	374 (67.1)
TZ	208 (37.3)
N stage, n (%)	
positive	8 (1.4)
Seminal Vesicle Invasion, n, (%)	48 (8.6)
Extracapsular Extension, n, (%)	138 (24.8)
Resection Margins, n, (%)	169 (30.3)
PSA Recurrence, n, (%)	66 (11.8)

PSA = prostate-specific antigen; T stage = tumor stage; N stage = lymph node stage; PZ = peripheral zone; TZ = transition zone.

3.2. Predictive Factors for Progression-Free Survival (PFS)

The ROC curve was used to calculate the relationship between BCR and tumor volume, and the optimal cutoff value was identified as 2.8 cc (AUC = 0.69) (Supplementary Figure S1A). We analyzed different tumor volume cutoff values (0.5 cc, 1.0 cc, 2.0 cc, 2.8 cc, 3.0 cc, 3.5 cc) and compared HR and *p*-values. The results confirmed that 2.8 cc is the optimal cut-off value as a

predictive factor for BCR (Table 2). (The cutoff values of two tumor volumes with $p < 0.0001$ that were not selected (3.0 cc and 3.5 cc) were also verified by corresponding models, as shown in Supplementary Figures S2 and S3).

Table 2. Univariable and multivariable cox proportional hazard regression models in predictive factors for PFS in localized Pca (overall risk).

		Univariable			Multivariable		
	Cut Off	**HR**	**95% CI**	***p* Value**	**HR**	**95% CI**	***p* Value**
Age	≥67	0.96	0.59–1.57	0.8842			
initial PSA	≥7.71 ng/mL	1.65	1.00–2.73	0.0505			
PSAD	≥0.26	2.06	1.21–3.53	0.0082	1.51	0.73–3.09	0.2643
GS	≥7	1.15	0.46–2.88	0.7593			
T stage	≥T3	4.66	2.81–7.73	<0.0001	1.69	0.77–3.71	0.1894
RM	positive	4.18	2.46–7.10	<0.0001	1.99	0.94–4.20	0.0712
Tumor location							
	Apex	1.45	0.70–3.02	0.3166			
	PZ	3.28	1.01–10.60	0.0472	2.21	0.49–10.05	0.3030
	posterior	2.24	1.07–4.65	0.0314	1.72	0.72–4.12	0.2193
TV							
	≥0.5 cc	1.61	0.73–3.53	0.2344			
	≥1.0 cc	2.18	1.11–4.27	0.0240			
	≥2.0 cc	2.74	1.55–4.82	0.0005			
	≥2.8 cc **	3.10	1.86–5.17	<0.0001	2.47	1.14–5.36	0.0225 *
	≥3.0 cc	2.96	1.80–4.88	<0.0001			
	≥3.5 cc	2.80	1.72–4.58	<0.0001			

PSA = prostate-specific antigen; PSAD = prostate-specific antigen density; GS = Gleason score; T stage = tumor stage; RM = resection margins; HR = hazard ratio; CI = confidence interval; * *p*-value < 0.05, ** tumor volume cutoff value based on the ROC curve.

Univariate and multivariate predictors for BCR obtained from cox proportional hazard analysis are shown in Table 2. The predictors for BCR were pathological stage T ≥ 3 (HR = 4.66 [95% CI: 2.81–7.73], $p < 0.0001$), positive surgical margin (HR = 4.18 [95% CI: 2.46–7.10], $p < 0.0001$), tumor volume ≥ 2.8 cc (HR = 3.10 [95% CI: 1.86–5.17], $p < 0.0001$), followed by PSA density ≥0.26 (HR = 2.06 [95% CI: 1.21–3.53], $p = 0.0082$), tumor located in the Posterior region (HR = 2.24 [95% CI: 1.07–4.65], $p = 0.0314$), tumor located in the PZ (HR = 3.28 [95% CI: 1.01–10.6], $p = 0.0472$). The multivariate analysis showed that the independent predictor of BCR was only tumor volume ≥ 2.8 cc (HR = 2.47 [95% CI: 1.14–5.36], $p = 0.0225$) (Table 2).

The Kaplan–Meier method was used to evaluate the PFS curve. The PFS of patients with tumors located in the PZ was inferior to those in the TZ (Figure 2A $p = 0.0354$). Furthermore, patients harboring tumors located in the posterior had shorter PFS than those in the anterior area (Figure 2B $p = 0.027$). Consistent with cox analysis, there was no significant difference between the PFS of the patients with tumors in the apex and not-apex area (Figure 2C $p = 0.3135$). PFS in the patients with tumor volume ≥ 2.8 cc was significantly inferior to those with less than 2.8 cc (Figure 2D $p < 0.0001$).

3.3. Model for Predicting PFS by Tumor Volume at Specific Location

Based on the analysis of clinical factors related to BCR in Table 2 and Figure 2, tumor volume and tumor location (PZ and Posterior location) were statistically significant predictive factors. Therefore, we established a risk classification model using tumor volume and location to stratify patients on the basis of risk of progression. The three risk factors that predict BCR in the model are tumor volume ≥ 2.8 cc, tumor located in PZ, and tumor located in the posterior area. The capability of the unfavorable risk to predict BCR was

independent predictor of BCR was only tumor volume ≥ 2.8 cc (HR = 2.47 [95% CI: 1.14–5.36] $p = 0.0225$) (Table 2).

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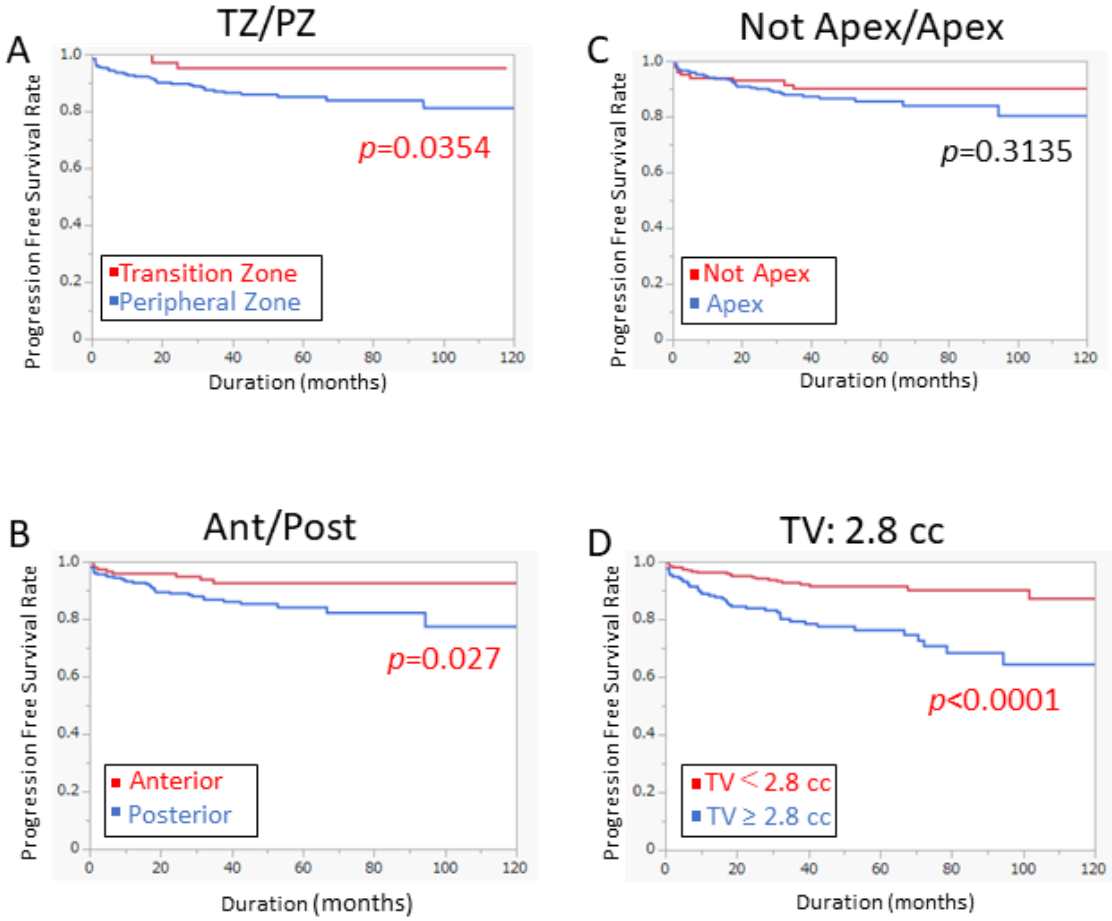

Figure 2. Prognostic significance of tumor location and tumor volume. (**A**) Patients with tumor in the PZ had significantly worse PFS than those in the TZ ($p = 0.0354$). (**B**) Patients with tumor in the posterior region had significantly worse PFS than those in the anterior region ($p = 0.027$). (**C**) There was no difference in PFS between apex and non-apex regions. (**D**) Patients with tumor volume ≥ 2.8 cc had significantly worse PFS than those <2.8 cc ($p < 0.0001$).

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Table 3. Univariable and multivariable cox proportional hazard regression models in predictive factors for PFS in localized Pca (overall risk) with unfavorable risk.

		Univariable			Multivariable		
Cut Off		**HR**	**95% CI**	***p* Value**	**HR**	**95% CI**	***p* Value**
Age	≥67	0.96	0.59–1.57	0.8842	-	-	-
initial PSA	≥7.71 ng/mL	1.65	1.00–2.75	0.0505	-	-	-
PSAD	≥0.26	2.06	1.21–3.53	0.0082	1.55	0.76–3.15	0.2307
GS	≥7	1.15	0.46–2.88	0.7593	-	-	-
T stage	≥T3	4.66	2.81–7.73	<0.0001	1.64	0.74–3.65	0.2261
RM	positive	4.18	2.46–7.10	<0.0001	2.09	0.99–4.42	0.0548
Unfavorable Risk	PZ + Post + TV2.8 cc	4.74	2.60–8.65	<0.0001	3.16	1.52–6.56	0.0020 *

PSA = prostate-specific antigen; PSAD = prostate-specific antigen density; GS = Gleason score; T stage = tumor stage; RM = resection margins; PZ + Post + TV2.8 cc = tumor volume ≥ 2.8 cc in posterior location of peripheral zone; HR = hazard ratio; CI = confidence interval; * p-value < 0.05.

To further explore the predictive ability of the novel risk model, we divided the patients into the low-risk group, intermediate-risk group, and high-risk group according to

the risk grouping of Pca by the American Cancer Society (ACS) [20] and validated the predictive value of the risk models among different ACS risk groups. In the analysis of the high-risk group, our unfavorable risk model could not predict disease progression independently (Table 4). However, the risk factors were the only independent predictor for PFS among patients with low to intermediate-risk groups (HR 4.43 [95% CI: 1.51–13.01], $p = 0.0068$) (Table 5).

Table 4. Univariable and multivariable cox proportional hazard regression models in predictive factors for PFS in localized Pca (high risk).

	Univariable				Multivariable		
	Cut Off	**HR**	**95% CI**	***p* Value**	**HR**	**95% CI**	***p* Value**
Age	≥67	0.76	0.40–1.47	0.4167	-	-	-
initial PSA	≥7.71 ng/mL	1.04	0.52–2.08	0.9097	-	-	-
PSAD	≥0.26	1.9	0.82–4.40	0.1326	-	-	-
GS	≥7	1.29	0.18–9.46	0.7991	-	-	-
T stage	≥T3	4.38	2.11–9.10	<0.0001	1.98	0.75–5.25	0.1701
RM	positive	4.65	2.16–10.02	<0.0001	2.37	0.95–5.91	0.0649
Unfavorable Risk	PZ + Post + TV2.8 cc	3.5	1.64–7.47	0.0012	1.87	0.77–4.53	0.1653

PSA = Prostate Specific Antigen; PSAD = Prostate Specific Antigen Density; GS = Gleason Score; T stage = Tumor Stage; RM = Resection Margins; HR = Hazard Ratio; CI = Confidence Interval.

Table 5. Univariable and multivariable cox proportional hazard regression models in predictive factors for PFS in localized Pca (low to intermediate risk).

	Univariable				Multivariable		
	Cut Off	**HR**	**95% CI**	***p* Value**	**HR**	**95% CI**	***p* Value**
Age	≥67	1.07	0.51–2.25	0.8546	-	-	-
initial PSA	≥7.71 ng/mL	1.56	0.74–3.28	0.2458	-	-	-
PSAD	≥0.26	1.52	0.72–3.19	0.2716	-	-	-
GS	≥7	0.74	0.26–2.15	0.5855	-	-	-
T stage	≥T3	3.34	1.59–7.01	0.0015	0.97	0.28–3.38	0.961
RM	positive	3.03	1.42–6.47	0.0043	1.38	0.43–4.41	0.5904
Unfavorable Risk	PZ + Post + TV2.8 cc	4.71	1.75–12.69	0.0022	4.43	1.51–13.01	0.0068 *

PSA = prostate-specific antigen; PSAD = prostate-specific antigen density; GS = Gleason score; T stage = tumor stage; RM = resection margins; HR = hazard ratio; CI = confidence interval; PZ + Post + TV2.8 cc = tumor volume ≥ 2.8 cc in posterior location of the peripheral zone. * *p*-value < 0.05.

3.4. Risk Model to Stratify Patient Prognosis

According to our established risk model, we divided the patients into three groups (favorable; displayed zero risk factors, moderate; displayed one or two risk factors, unfavorable; displayed all three risk factors). Overall, 61, 343, and 104 patients were classified as belonging to the favorable, moderate, and unfavorable group, respectively (Figure 3A).

The PFS curves of the three groups of patients (Figure 3B) showed that the PFS of the unfavorable group was significantly worse than that of the moderate group ($p < 0.0001$) and the favorable group ($p = 0.001$), while there was no significant difference between the moderate group and the favorable group ($p = 0.1150$).

The median tumor volume of the three groups was 1.33 cc, 1.81 cc, and 4.92 cc, respectively and there were significant differences between the three groups (Figure 3C).

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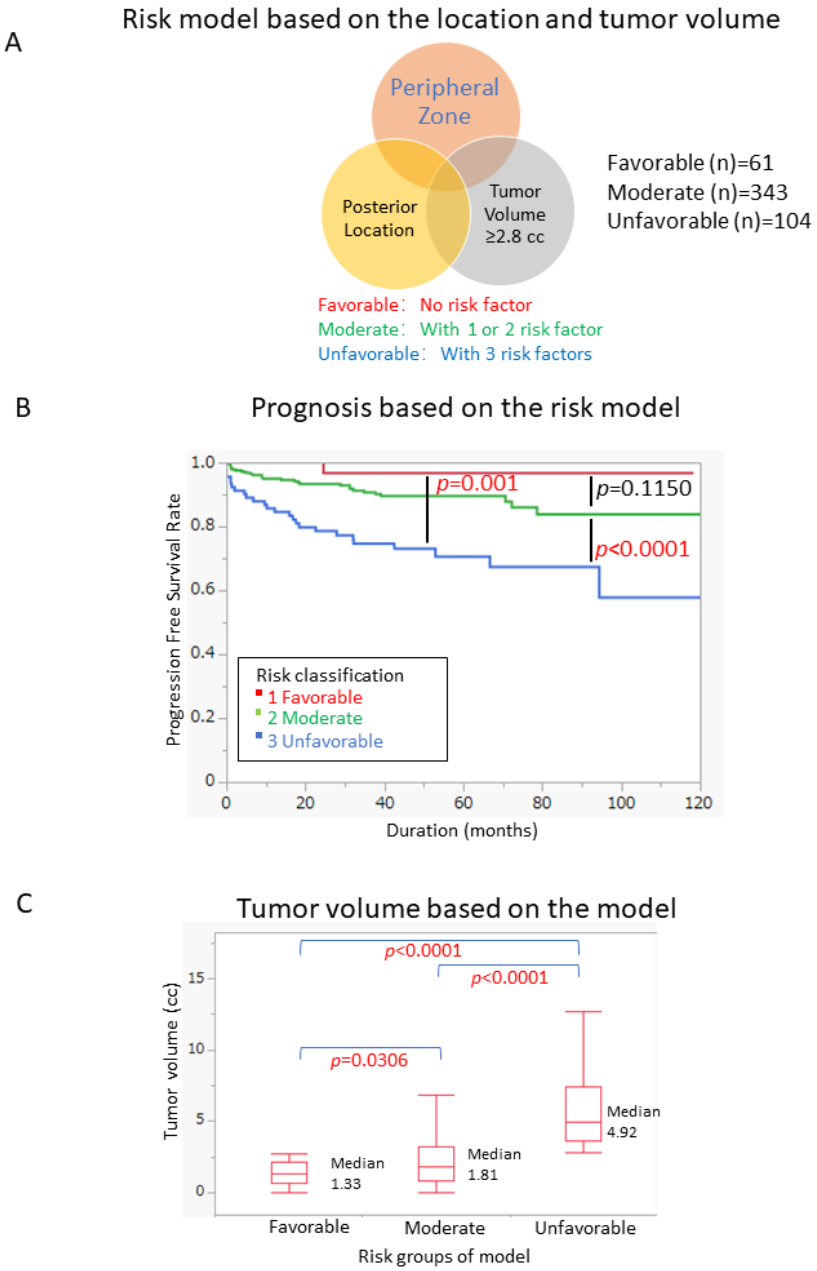

Figure 3. Prognostic model based on the location and tumor volume (**A**) Venn diagram of risk model based on the location and tumor volume. (**B**) Risk classification significantly differentiated the PFS between the Favorable and Unfavorable group ($p < 0.0001$) and the Moderate and Unfavorable group ($p < 0.0001$). (**C**) The tumor volume showed significant differences among different risk groups.

In addition, we analyzed the impact of tumor volume on PFS in different prostate regions with the tumor volume of 2.8 cc as the threshold (Figure 4). The results suggested that the PFS of tumor $\geq$ 2.8 cc in the PZ is significantly worse than that of less than 2.8 cc (Figure 4A $p < 0.0001$). Similar results were observed for tumors $\geq$ 2.8 cc in the posterior location (Figure 4C $p < 0.0001$). Of note, the 2.8 cc cutoff value in TZ also showed a significant difference in PFS between the two groups (Figure 4B $p = 0.0345$). On the other hand, the significant difference was not seen in the anterior area (Figure 4D $p = 0.0873$).

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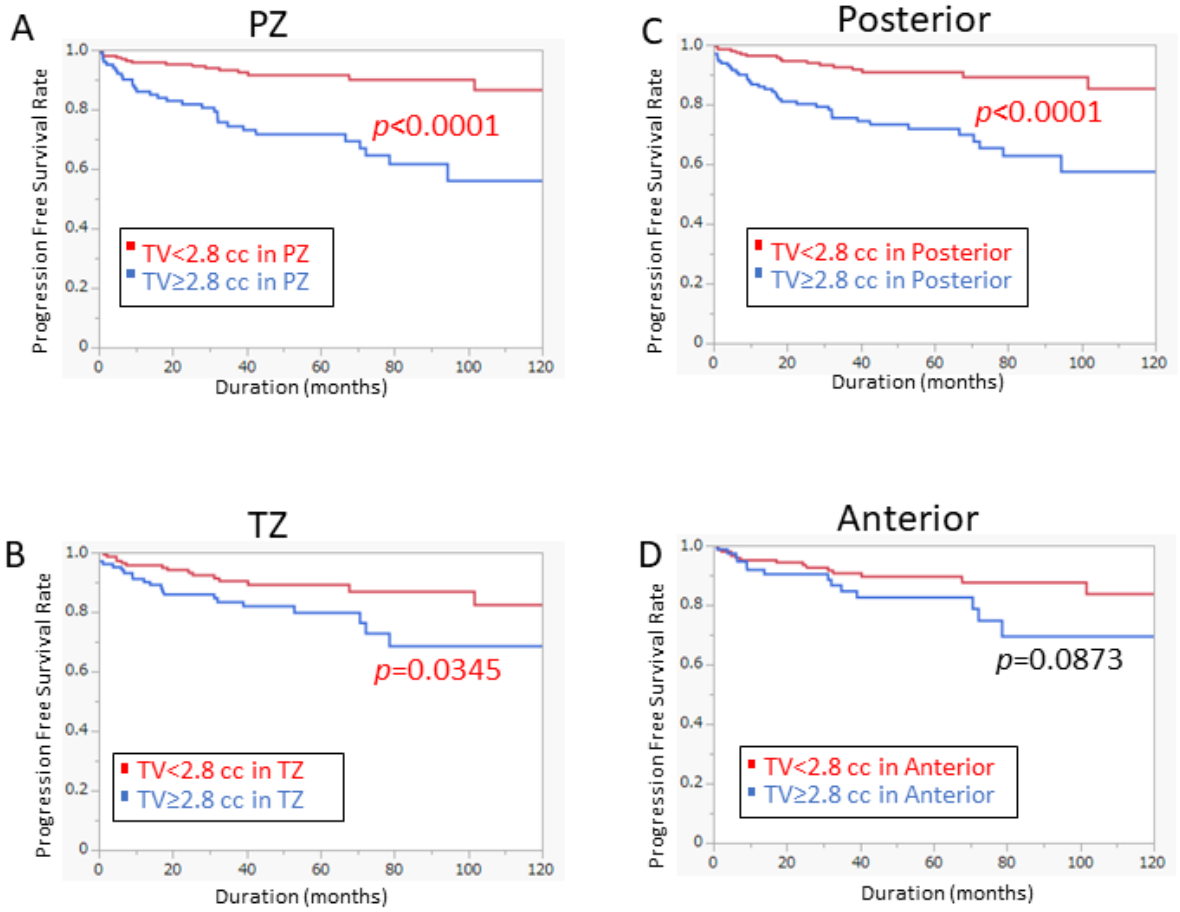

Figure 4. Prognostic significance of Tumor volume 2.8 cc based on the location. (**A**) Patients with tumor volume ≥ 2.8 cc had significantly worse PFS in the PZ ($p < 0.0001$). (**B**) Patients with tumor volume ≥ 2.8 cc had significantly worse PFS in the TZ ($p = 0.0345$). (**C**) Patients with tumor volume ≥ 2.8 cc had significantly worse PFS in the posterior region ($p < 0.0001$). (**D**) In the anterior region, there was no difference in PFS by tumor volume cutoff of 2.8 cc.

4. Discussion

In our study, a tumor with a volume ≥ 2.8 cc was identified as an independent predictive factor for BCR ($p = 0.0225$). Furthermore, we established novel risk classification together with PZ and posterior location, which distinguished PFS between different risk groups. We believe this risk model will provide novel prognostic significance in patients who received RP.

Previous studies showed the positive surgical margin after RP is a potential predictive factor for BCR [21–29]. It is difficult to completely avoid the incidence of positive surgical margins through objective methods. Several studies found that positive surgical margins with limited length [30,31], locations [32], or quantity [33] decreased the correlation with BCR. Another study showed that tumor volume was associated with BCR in patients who underwent RP with negative surgical margins [34]. In addition, tumor volume and GS were even more significant predictors for BCR than positive margins [35] and the location of the tumor could predict the incidence of positive surgical margins [36–39]. Multivariate analysis showed that the predictive value of our risk model was superior to the positive surgical margin. These findings suggested that focusing on tumor volume and location, not only resection margins will give us better prognostic information in the treatment of localized Pca.

Regarding the prognostic significance of tumor localization, tumors originating in the TZ have been reported to be associated with a better prognosis in comparison with those

in the PZ [39–41]. Augustin et al. found that the location of prostate cancer in the TZ was associated with better progression-free survival after RP (p = 0.0402) [40]. However, the zonal location offers no advantage over the well-established prognostic factors in predicting recurrence. Some more detailed anatomical differentiation (anterior, posterior, the apex of prostate, bladder neck) also revealed the difference in tumor location on prognosis [42,43]. Magheli et al. found that tumors in the anterior prostate were associated with favorable pathological features and improved biochemical-free survival, although it was not an independent predictor of BCR [42]. There are also some studies that have concluded that tumor location is not related to prognosis [44,45].

Tumor volume has been reported to show a significant correlation with BCR after RP [46–50]. Generally, tumor volume < 0.5 cc has been considered as an insignificant Pca, which has a low potential of recurrence [51]. The predictive factors for BCR in patients with low-volume prostate cancer ($\leq$0.5 cc) have not been well studied [52]. Several reports proposed to increase the thresholds of volume for insignificant cancer to avoid over-treatment [14], however, other studies showed that the modified criteria had a higher risk of BCR in Gleason 4/5 cancer [53]. The tumor volume was superior to the percentage of cancer (tumor volume/prostate volume ratio) for predicting the prognosis after RP [54]. Different tumor volume cut-off values were proposed to determine the prognosis of Pca. Friedersdorff et al. suggested that tumor volume $\geq$ 5 cc (AUC = 0.79) was a significant prognostic factor for BCR [55]. Another study set the cut-off values as: minimal ($\leq$1.0 cc), middle (1.1–5.0 cc), or extended (>5.0 cc) [47]. Shin et al. divided the tumor volume into three groups according to 2 cc and 5 cc, in multivariate analysis, recurrence-free survival could be independently predicted [56]. The tumor volume in the surgical specimen after neoadjuvant therapy was investigated and the study showed that patients with residual tumors $\geq$ 1.0 cc in the specimen had a higher risk of BCR [57]. Raison et al. studied 685 British patients who underwent laparoscopic and robot-assisted RP and revealed that 2.5 cc (AUC = 0.71) was the best cutoff value for predicting BCR [58]. Of note, some studies showed that the tumor volume alone may not be able to evaluate the prognosis of recurrence and prognosis after RP [13,59]. O'Neil et al. suggested that tumors in some locations are larger and more likely to invade the sites that are prone to recurrence [37]. However, there have been no studies that have analyzed the prognostic value of tumor volume combined with tumor localization.

In our study, we attempted to evaluate the potential interaction between tumor volume and location, the tumor volume cutoff value obtained by the ROC curve was 2.8 cc (AUC = 0.69). Therefore, we used the tumor volume threshold ($\geq$2.8 cc) of the specific location to improve the capability of our risk model. We hypothesized that the larger tumor volume in the PZ and/or posterior of the prostate may be associated with BCR. Our findings demonstrated that the prognostic significance of tumor volume over 2.8 cc varied by tumor localization (Figure 4). In our model, the interaction between prostate tumor location and volume was a promising predictor of prostate BCR. Interestingly, our risk model was an independent predictor in patients with low and intermediate risk while it was not in patients with high risk. Extended dissection during surgery and close follow-up after surgery may enhance clinical benefit in patients who met our criteria.

The limitations of this study are as follows. First, our study included a single Asian race. Compared with the western population, the Asian population has a lower incidence and mortality of prostate cancer [60]. The tumor volume of African American men with prostate cancer is larger than that of white men [61]. The risk of BCR in black Americans has been reported to be 1.6 times higher than that in white Americans [62]. These results suggested that there may be differences in clinical and pathological features between races. Further validation of our risk model will be warranted in other patients' cohorts. Second, our study may need to be further investigated using genomic analysis. The previous study has revealed that prostate cancer risk alleles are associated with prostate cancer volume and prostate size [63]. Downregulation of PAH and AOC1 and upregulation of DDC, LIN01436, and ORM1 were associated with the development of prostate cancer [8,64].

Molecular and cellular biological studies are also closely related to the site of prostate tumorigenesis [41]. Studying the specific genes behind it could improve understanding of the region or cell-type characteristics of prostate cancer. These features account for differences in tumor progression and invasion between different regions of the prostate [41]. The unique biological characteristics of tumor types in different prostate regions can help guide individualized treatment and patient risk stratification. Finally, further validation of our clinical parameters using the latest imaging system PSMA/PET [65] or artificial intelligence system (deep learning) [66] may enhance the clinical importance of this study.

5. Conclusions

Tumor volume $\geq$ 2.8 cc was an independent predictive factor for BCR in patients who received RP. Furthermore, we established a novel risk model using tumor volume over 2.8 cc and tumor location (PZ and/or posterior). Our risk classification could predict patient prognosis and will help us to optimize peri-operative and post-operative treatment strategies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cancers14235823/s1, Figure S1: (A) Tumor volume cut off based on the ROC curve. (B) Tumor volume based on the location; Figure S2: (A) A supplemental model that included the 3.0 cc tumor volume as one of the factors in the risk model. (B) Risk classification significantly differentiated the PFS between the Favorable and Unfavorable group ($p = 0.0008$) and the Moderate and Unfavorable group ($p < 0.0001$); Figure S3: (A) A supplemental model that included the 3.5 cc tumor volume as one of the factors in the risk model. (B) Risk classification significantly differentiated the PFS between the Favorable and Unfavorable group ($p = 0.0001$) and the Moderate and Unfavorable group ($p < 0.0001$).

Author Contributions: H.B. contributed to collecting data, preparing figures, and writing; S.S. and X.Z. contributed to analyzing data, collecting bibliography, drawing tables, and writing; Y.Y. and J.R. contributed to analyzing data; A.F., M.K., N.T., T.S., Y.I. and K.A. contributed to collecting data; S.S. and T.I. contributed to the supervision of all the activities; The first draft of the manuscript was prepared by H.B. and X.Z. performed subsequent amendments. S.S. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The present study was supported by grants from Grant-in-Aid for Scientific Research (C) (20K09555 to S.S.), Grant-in-Aid for Scientific Research (B) (20H03813 to T.I.), and the Japan China Sasakawa Medical Fellowship (to X.Z.).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Chiba University of Graduate School of Medicine and School of Medicine (protocol code 1768 and date of approval 1 March 2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Abbreviations

Pca	prostate cancer
RP	radical prostatectomy
BCR	biochemical recurrence
CRPC	castration-resistant prostate cancer
PSA	prostate-specific antigen
PZ	peripheral zone

TZ	transition zone
CZ	central zone
TV	tumor volume
GS	Gleason score
ROC	Receiver Operating Characteristic
AUC	Area Under the Curve
PFS	Progression-Free Survival
ACS	American Cancer Society

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Journal of Pharmacological Sciences 150 (2022) 251–258

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full paper

Targeting L-type amino acid transporter 1 in urological malignancy: Current status and future perspective

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ARTICLE INFO

Article history:
Received 9 September 2021
Received in revised form
18 September 2022
Accepted 3 October 2022
Available online 7 October 2022

Keywords:
LAT1
4F2hc
Prostate cancer
Renal cancer
Bladder cancer

ABSTRACT

Amino acid transporters are responsible for the uptake of amino acids, critical for cell proliferation. L-type amino acid transporters play a major role in the uptake of essential amino acids. L-type amino acid transporter 1 (LAT1) exerts its functional properties by forming a dimer with 4F2hc. Utilizing this cancer-specificity, research on diagnostic imaging and therapeutic agents for malignant tumors targeting LAT1 progresses in various fields. In hormone-sensitive prostate cancer, the up-regulation of L-type amino acid transporter 3 (LAT3) through the androgen receptor (AR) has been identified. On the other hand, in castration-resistant prostate cancer, the negative regulation of LAT1 through AR has been determined. Furthermore, 4F2hc: a binding partner of LAT1, was identified as the specific downstream target of Androgen Receptor Splice Variant 7: AR-V7. LAT1 has been suggested to contribute to acquiring castration resistance in prostate cancer, making LAT1 a completely different therapeutic target from anti-androgens and taxanes. Increased expression of LAT1 has also been found in renal and bladder cancers, suggesting a contribution to acquiring malignancy and progression. In Japan, clinical trials of LAT1 inhibitors for solid tumors are in progress, and clinical applications are now underway. This article will summarize the relationship between LAT1 and urological malignancies.

1. Introduction

Living organisms require substances in order to function and, ultimately, to sustain life. In particular, the organism needs to take in essential nutrients such as carbohydrates, fats, and proteins from the outside. Proteins are metabolized into amino acids via peptides by digestive enzymes, and amino acid molecules are absorbed through the epithelial mucosa of the small intestine and transferred to the bloodstream, where they undergo various metabolic processes. The mechanism responsible for this transport is the amino acid transporter, which contributes to maintaining biological functions. Amino acid transporters play a role in maintaining cell survival by transferring amino acids, which are essential for living organisms, into cells and maintaining tissue specificity by transporting them in a particular direction.[1] The amino acids taken up are used for protein biosynthesis, activation of the mTOR (mammalian target of rapamycin) pathway, an important signal for cell growth and proliferation, and maintenance of redox reactions and homeostasis in cells.[2,3]

Amino acid transporters can be broadly classified into the ABC (ATP binding cassette) family and SLC (solute carrier) family. The

Abbreviations: ABC, ATP binding cassette; AR, androgen receptor; AR-V7, androgen receptor splicing variant 7; BAT, b0 amino acid transporter; CCRC, clear cell renal cell carcinoma; CRPC, castration resistant prostate cancer; DHT, dihydrotestosterone; GC, gemcitabine/cisplatin combined chemotherapy; HAT, heterodimeric amino acid transporters; HSPC, hormone sensitive prostate cancer; LAT, L-type amino acid transporter; mTOR, mammalian target of rapamycin; mTORC, mammalian target of rapamycin complex; MVAC, methotrexate/vinblastine/doxorubicin/cisplatin combined chemotherapy; PSMA, prostate specific membrane antigen; SLC, solute carrier.

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Peer review under responsibility of Japanese Pharmacological Society.

https://doi.org/10.1016/j.jphs.2022.10.002

ABC family transports ATP by co-benefiting and utilizing its energy and 7 subgroups have been identified in humans. The SLC (solute carrier) family transports without conjugating with ATP. L-type amino acid transporter 1: LAT1 (*SLC7A5*), which is the subject of this paper, can be classified as a solute carrier: SLC, and SLCs are further classified into families with different exchange substrates and localization and perform various functions.

Metabolism occurs in cancer cells as it does in normal tissues, but more nutrients may be required to compensate for changes in the surrounding environment and the frequency of cell growth. In Japan, a phase II clinical trial of JPH203, a selective inhibitor of LAT1, is underway in biliary tract cancer.

In this article, we report the physiological functions of amino acid transporters and their relevance to malignancies, especially urological cancers, focusing on LAT1, which is upregulated in malignant tumors.

2. Biological functions of LATs amino acid transporter and cancer

LAT1 is an ATP-independent, sodium-independent, 12-fold transmembrane amino acid transporter belonging to the SLC7 family. It has been reported to cause decreased leucine uptake and cell proliferation in LAT1 knockout cells.[4] Therefore, it is thought that the exchange substrate transports a wide range of essential amino acids, mainly leucine.[5–9]

The SCL7 family described above consists of L-type amino acid transporters: LAT (*SLC7A5-13*, *SLC7A15*) and cationic amino acid transporters: CAT (*SLC7A1-4*, *SLC7A14*). Among these, LAT forms a heterodimeric amino acid transporter complex: HATC and constitutes its light subunit.[10–16]

LAT1 (17), L-type amino acid transporter 3: LAT3 (*SLC43A1*),[18] and system ASC transporter 2: ASCT2 (*SLC1A5*)[19] have been reported to be upregulated in tumor cells.[20–22] Gastrointestinal malignancies, breast cancer, prostate cancer, renal cancer, bladder cancer, lung cancer, glioma, endometrial cancer, and pancreatic cancer, have been identified to express a high level of LAT1[4,6,7,23–30] (Table 1).

Leucine, a major exchange substrate of LAT, is an essential amino acid and one of the signal regulators of mTORC1 (mammalian target of rapamycin complex 1). mTORC1 is known to regulate mRNA translation,[31] ribosome biogenesis by regulating rRNA transcription,[32] and autophagy, and thus plays a role in regulating protein synthesis and cell proliferation.

LAT3 and LAT4 have been found to have fewer exchange substrates than LAT1 and LAT2 (18, 33) (Table 1).

3. Heterodimeric amino acid transporters

Some members of the SLC7 family form heterodimeric amino acid transporters (HATs). These include a 14-transmembrane cationic amino acid transporter and a 12-transmembrane heterodimeric amino acid transporter.[1,34] LAT1 is disulfide-linked to the SLC3 family members 4F2hc (4F2 heavy chain: *SLC3A2*). BAT1(SLC7A9) is disulfide-linked to rBAT (related to b0 amino acid transporter: *SLC3A1*)[35] (Fig. 1). Heterodimeric amino acid transporters have the structural feature of being composed of a light subunit and a heavy subunit, which form a dimer through disulfide bonds. This morphological feature is thought to enable the localization of LAT1 on the plasma membrane, which cannot be achieved by LAT1 alone. As an example, it has been reported that in the absence of 4F2hc, LAT1 exists inside the cell, but in the presence of 4F2hc, it moves to the cell surface by forming HATs.[36]

Although LAT1 and L-type amino acid transporter 2: LAT2 can transport leucine by themselves, their substrate affinity and specificity have also been found to be regulated by 4F2hc.[37] Because of this property, HATs are also thought to be involved in the pathogenesis of aminoaciduria (cystinuria, lysinuria), tumor cell proliferation, and glial tumor invasion[18,30,34,35,38,39] (Table 2).

4. Relationship between LAT1 and LAT2

Both LAT1 and LAT2 are sodium-independent amino acid transporters that form HATs through disulfide bonds with 4F2hc. LAT1 and LAT2 have similar functions due to the commonality of their exchange substrates, but LAT2 is thought to have a broader range of exchange substrates. In addition, LAT2 is distributed in the proximal tubules of kidneys and small intestinal epithelium and is involved in the uptake and reabsorption of amino acids from the body.[40,41] On the other hand, the expression of LAT1 in normal tissues can be observed in the brain, testis, and placenta.[17,42,43] Although LAT2 is commonly expressed in the brain, testis, and placenta, it is also involved in the absorption and reabsorption of amino acids, suggesting that LAT2 is mainly responsible for the uptake of amino acids from outside the body and LAT1 is mainly responsible for the uptake of amino acids into specific cells.

In another aspect, LAT1 is known to be upregulated in various tumor cells and has been reported to be a poor prognostic factor, while LAT2 has been reported to be less distributed in malignant tumors and more distributed in normal tissues. Therefore, it is possible that LAT1 and LAT2 have a tumor type and a normal tissue type property, respectively[17] (Fig. 2).

5. LAT1 and 4F2hc characteristics

Tumor cells require increased uptake of glucose and amino acids for their biosynthesis, related to their rapid growth and changes in the surrounding environment.[44] In amino acids, increased expression of amino acid transporters in tumor cells has been observed in various cancer types.

The exchange substrate of LAT1 is an essential amino acid, and when a single molecule of amino acid is taken into the cell, glutamine is transported out of the cell instead.[45] However, since glutamine is required for ATP production in tumor cells, high expression of ASCT2 (*SLC1A5*), a sodium-dependent neutral amino acid transporter that can take glutamine into the cell, allows tumor cells maintenance of intracellular glutamine levels.[20] This glutamine is used for ATP production and prevents the depletion of the exchange substrate of LAT1 (Fig. 3).

4F2hc functions as a heavy subunit of the transporter complex and plays a role in the localization and stabilization of LAT1 on the plasma membrane and as an enhancer of integrin signaling.[11,45–50] 4F2hc deficiency results in the loss of intracellular amino acid pools, including leucine and arginine, which are active factors of mTOR kinase,[48,51] and increased oxidative stress, DNA damage, and radiosensitivity in head and neck squamous cell carcinoma cells.[11]

6. Application of LAT1 to diagnostic imaging

The increased expression of LAT1 in tumor cells has been studied for the detection of malignant tumors by imaging diagnosis. FDG-PET, which is currently used in clinical practice, is an imaging diagnosis in which the glucose that tumor cells consume in large amounts is radiolabeled (18F-FDG: 18-fludeoxyglucose) the presence or absence of accumulation is confirmed. However, the accumulation of FDG is not specific to malignant tumors but also occurs in areas with high physiological accumulation, such as the brain, and inflammatory cells, making differential diagnosis often tricky. In evaluating malignant tumors of the urinary tract, FDG is excreted in the urine. Thus, hyperaccumulation around the urinary tract often masks

Table 1
LAT expression and function.

protein	gene	substance selectivity	expression pattern	subtype
LAT1[4,6,7,23-30]	*SLC7A5*	broad (Leu, Ile, Phe, Met, Tyr, His, Try, Val)	cancer testis brain ovary placenta spleen colon blood brain barrer fetal liver	system L1
LAT2[17,29,36,39,40-42]	*SLC7A8*	broad (Gly, Ala, Ser, Thr, Cys, Asn, Gln, Met, Leu, Ile, Val, Phe, Tyr, Trp, His)	kidney testis prostate small intestine lung heart spleen liver brain placenta ovary fetal liver muscle	system L1
LAT3[18,23,58]	*SLC43A1*	narrow (Leu, Ile, Val, Phe, Met)	prostate liver muscle kidney placenta	system L2
LAT4[33]	*SLC43A2*	narrow (Leu, Ile, Val, Phe, Met)	kidney placenta peripheral blood leukocytes small intestine	system L2

renal, pelvis, ureteral, bladder, and prostate cancers. In addition, the detection rate of prostate cancer is estimated to be even lower because of the weak accumulation of FDG.

Imaging diagnosis targeting amino acids instead of glucose is being studied. In 2016, the U.S. FDA approved FACBC PET labeled with 18-F on ACBC (Aminocyclobutane carboxylic acid), taken

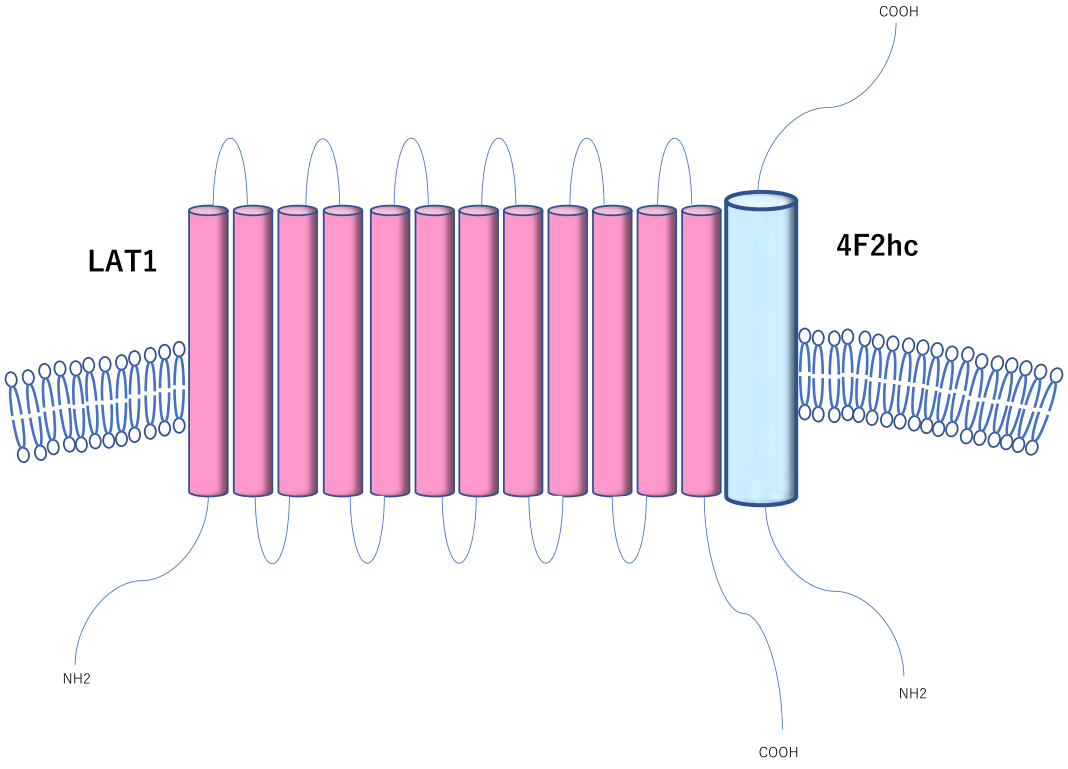

Fig. 1. Scheme shows the representation of LAT1/4F2hc complex. The structures of the LAT1 and 4F2hc heterodimers on the plasma membrane are shown in simplified form: LAT1 is a 12-fold transmembrane amino acid transporter that serves as the light chain of the dimer; 4F2hc is a single-fold transmembrane amino acid transporter that forms a dimer with LAT1 by disulfide bonds.

Table 2
Examples of heterodimeric amino acid transporters.

heavy chain (gene)	light chain	disease	expression pattern
rBAT (*SLC3A1*)[10,34]	b (0,+)-AT1 (*SLC7A9*)	cystinuria	kidney intestine liver pancreas
4F2hc (*SLC3A2*)[12,46,59]	LAT1 (*SLC7A5*) y + LAT2 (*SLC7A6*) y + LAT1 (*SLC7A7*) LAT2 (*SLC7A8*) ASC1 (*SLC7A10*) xCT (*SLC7A11*)	cancer	ubiquitous (depend on the light chain)

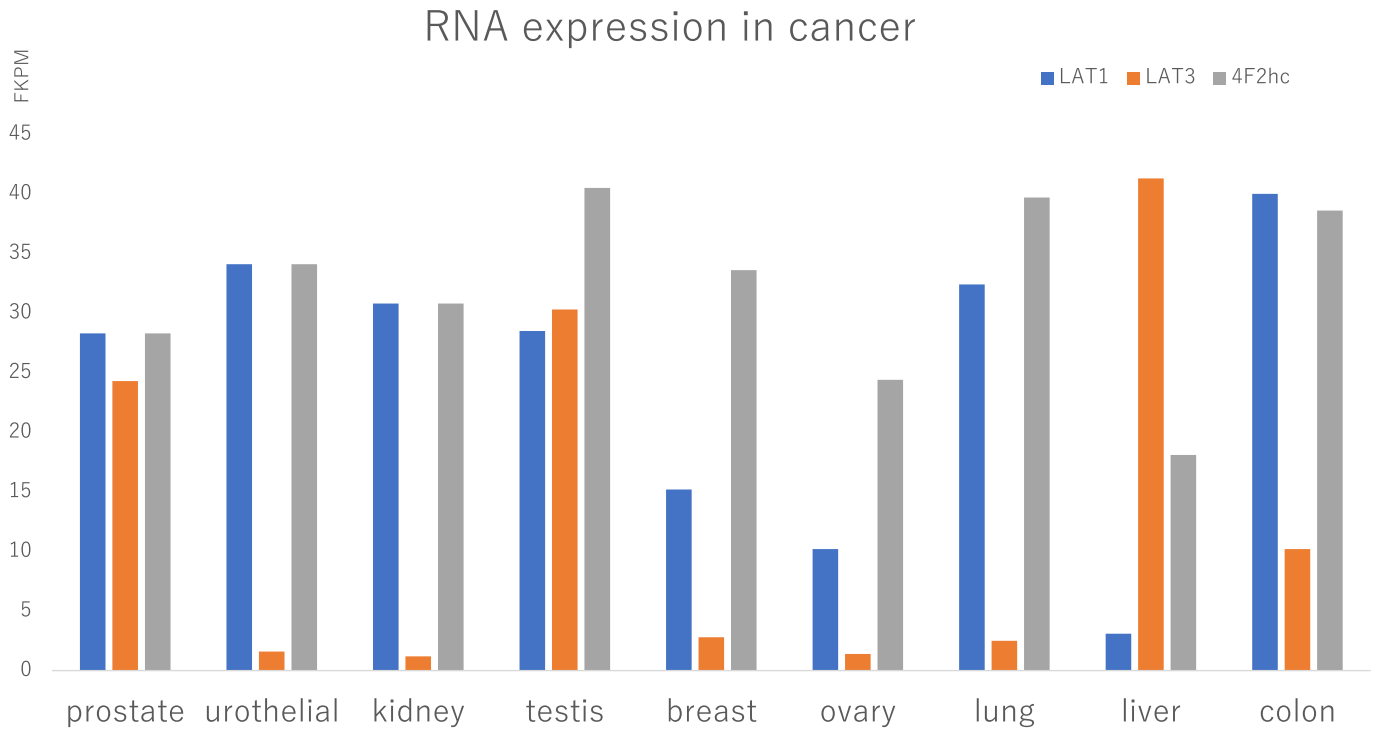

Fig. 2. The expression of LAT1, LAT3, and 4F2hc in tumor cells is shown, and LAT1 expression is more upregulated in many tumors than LAT3, which is the reason why LAT1 may be the tumor cell type amino acids transporter. However, in prostate and testicular cancers, LAT3 is expressed to the same extent. There may be differences in expression depending on hormone sensitivity in prostate cancer and histology in testicular cancer. Reference: the protein atlas (https://www.proteinatlas.org/).

into the body as an amino acid but is not metabolized intracellularly and does not degrade image quality, for the evaluation of recurrence of prostate cancer. Although FACBCs were expected to be taken up into cells by amino acid transporters, it was later found that their main bodies were actually LAT1 and ASCT2,[52] which is consistent with the increased expression of LAT1 in prostate cancer, as will be discussed later. It should also be noted that PSMA-PET, which uses ligands that bind to PSMA (prostate-specific membrane antigen), is applied in prostate cancer, but is entirely different from PET which targets amino acid transporters.

In addition, the construction of diagnostic imaging systems targeting LAT1, such as 18-FMT (18-F-α-methyltyrosine), which has higher malignancy specificity, has been proposed, and the usefulness of cancer-specific accumulation in patients with advanced lung cancer and esophageal cancer has been proposed.[53,54] Furthermore, 18F-FBPA (4-borono-2-18F-L-phenylalanine), which also targets LAT1, significantly reduced accumulation in inflammatory cells, which is often a problem with FDG, albeit in vitro.[55] In Japan, a phase I study of a nuclear medicine test targeting LAT1 using F-18 NKO-035, which is a PET probe with high selectivity for LAT1, has been completed. Its clinical application will be evaluated through further studies.

Thus, the high expression of LAT1 in tumor cells will enable new imaging modalities and may change the existing diagnostic protocols, such as FACBC PET in locally treated prostate cancer.

7. LAT1 targeting cancer therapy

As mentioned above, LAT1 is abundantly expressed in tumors and contributes significantly to the survival of tumor cells. Research on therapeutic drugs that target LAT1 for cancer control is also underway.

BCH (2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid) is a selective inhibitor of the L-type amino acid transporter and has been found to inhibit cell growth and proliferation by blocking leucine uptake into cells, as well as inducing apoptosis.[4] Triiodothyronine and thyroxine have also been reported to inhibit LAT1-mediated phenylalanine incorporation into cells.[56]

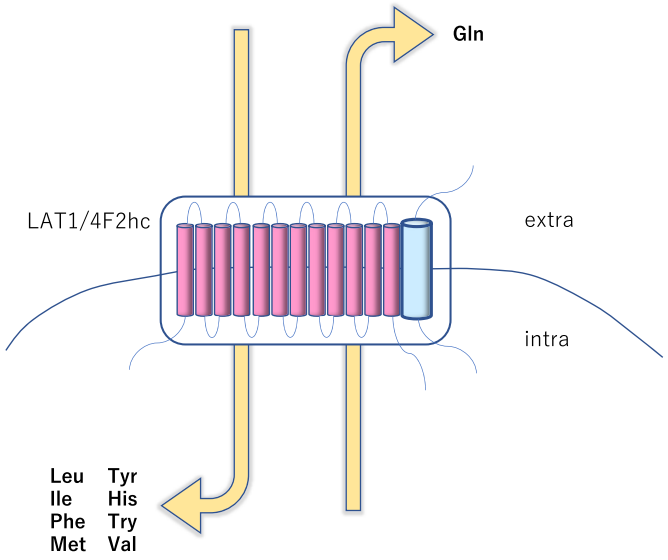

Fig. 3. Scheme shows the substances of LAT1/4F2hc. LAT1 is sodium-independent and transports a single molecule of glutamine out of the cell, and at the same time takes up large side-chain neutral amino acids such as leucine, histidine, methionine, isoleucine, valine, phenylalanine, tyrosine, and tryptophan into the cell.

Considering that LAT1 is more abundantly expressed in tumor cells, it can be expected that selective inhibition of LAT1 among L-type amino acid transporters will result in more minor damage to normal cells. JPH203 ((S)-2-amino-3-(4-((5-amino-2- phenylbenzo [d]oxazol-7-yl)methoxy)-3,5-dichlorophenyl)) is a selective inhibitor of LAT1 and has been reported to inhibit tumor cell growth in a concentration-dependent manner by inhibiting leucine uptake.[57] Phase I clinical trials of JPH203 for solid tumors have been completed in Japan, and its safety and tolerability have been confirmed. In addition, long-term responses in biliary tract cancer patients were confirmed in this study, and anti-tumor effects are expected.[58] A phase II study in biliary tract cancer is currently underway.

8. LAT1 and urological cancer

8.1. LAT1 in prostate cancer

There have been significant changes in the treatment of prostate cancer in recent years. In addition to primary hormone therapy, known as vintage hormone therapy, early administration of novel androgen receptor inhibitors for high-risk hormone-sensitive prostate cancer has been established. However, prostate cancer is known to progress to castration-resistant prostate cancer under androgen deprivation therapy, and the sequential therapy is still unclear.

LAT1 expression intensity is significantly correlated with the prognosis of prostate cancer patients and the Gleason score, and its potential as a biomarker for prostate cancer has been explored, and the relationship between prostate cancer and LAT1 has been clarified.[27] In addition, LAT1 expression is upregulated in castration-resistant prostate cancer cells compared to hormone-sensitive prostate cancer cells, and knockdown of LAT1 inhibits cancer cell proliferation, migration, and invasion.[8] In addition, in multivariate analysis, LAT1 expression has been reported to be an independent prognostic factor for castration-resistant prostate cancer.[8]

It has been reported that LAT3 expression is upregulated in hormone-sensitive prostate cancer cells before acquiring castration resistance.[59] Thus, it has been reported that androgen receptor (AR) increases LAT3 expression, while LAT3 expression is decreased and LAT1 expression is increased in castration-resistant prostate cancer that has acquired resistance after AR inhibition (Fig. 4).

As there are various factors, expression of androgen receptor splicing variant-7: AR-V7 leads hormone-sensitive prostate cancer to progress to castration-resistant prostate cancer under androgen deprivation therapy. One of the specific target genes of AR-V7 is *SLC3A2*, which encodes 4F2hc.[60] It suggests a link between the acquisition of castration resistance and the expression of the LAT1 coactivator.

Although there are various treatments for castration-resistant prostate cancer, they all have limited efficacy. In addition, drugs that target androgen receptors may cause early resistance to these drugs due to the increased expression of AR-V7.[61] The introduction of drugs that do not target the androgen receptor, such as the PARP inhibitor (Olaparib) for *BRCA* mutation-positive unresectable prostate cancer, a new drug for prostate cancer currently approved in Japan, may change the treatment of prostate cancer.

8.2. LAT1 in bladder cancer

The 5-year survival rate of stage IV bladder cancer is 19% (2012–2013 data of Japan), and it is not a malignant tumor with a good prognosis, so a therapeutic agent with an unprecedented mechanism of action is desirable.

In the treatment of advanced bladder cancer, cisplatin-based chemotherapy (GC: gemcitabine/cisplatin, MVAC: methotrexate/vinblastine/doxorubicin/cisplatin), which was introduced in the 1980s, is still used as the primary treatment until pembrolizumab, an anti-PD-1 antibody, was validated as second-line therapy in 2017.[62,63] Furthermore, in 2020, avelumab is approved as maintenance therapy for first-line treatment, and bladder cancer treatment is undergoing significant changes.[64]

In human bladder cancer tissues, LAT1 and 4F2hc are highly expressed compared to normal cells,[4,45] and siLAT1 and JPH203, a selective LAT1 inhibitor, inhibit cell proliferation, migration, and invasion in bladder cancer cell lines.[65] In addition, multivariate analysis showed a significant reduction in overall survival in cases with high expression of LAT1, which correlated with a higher grade of pathological T classification and tumor grade.[65] Furthermore, insulin-like growth factor-binding protein-5 (IGFBP-5) was identified as a downstream target of JPH203[65].

8.3. LAT1 in renal cancer

The kidney contains a variety of transporters that maintain physiological functions such as reabsorption and excretion. As for amino acid transporters, LAT2 is expressed in the proximal tubule and is responsible for the reabsorption of amino acids.[66]

Regarding the relationship between LAT1 and renal cancer, it has been found that the expression of mRNA in tumors of the clear cell renal cell carcinoma: CCRC is increased compared to normal tissues and that the expression of LAT2 and LAT3 is decreased compared to normal tissues.[23]

In a retrospective analysis, immunostaining of CCRC showed that the expression of LAT1 was increased compared to normal tissues,[67] and the overall survival rate and progression-free survival rate were significantly decreased in the group with high expression of LAT1 (23, 67). It was also reported that JPH203 decreased the leucine uptake rate of renal cancer cells and inhibited cell proliferation, migration, and invasion in renal cancer cell lines.[67]

An association between LAT1 mRNA expression and malignancy has also been suggested, with the highest expression in grade 3

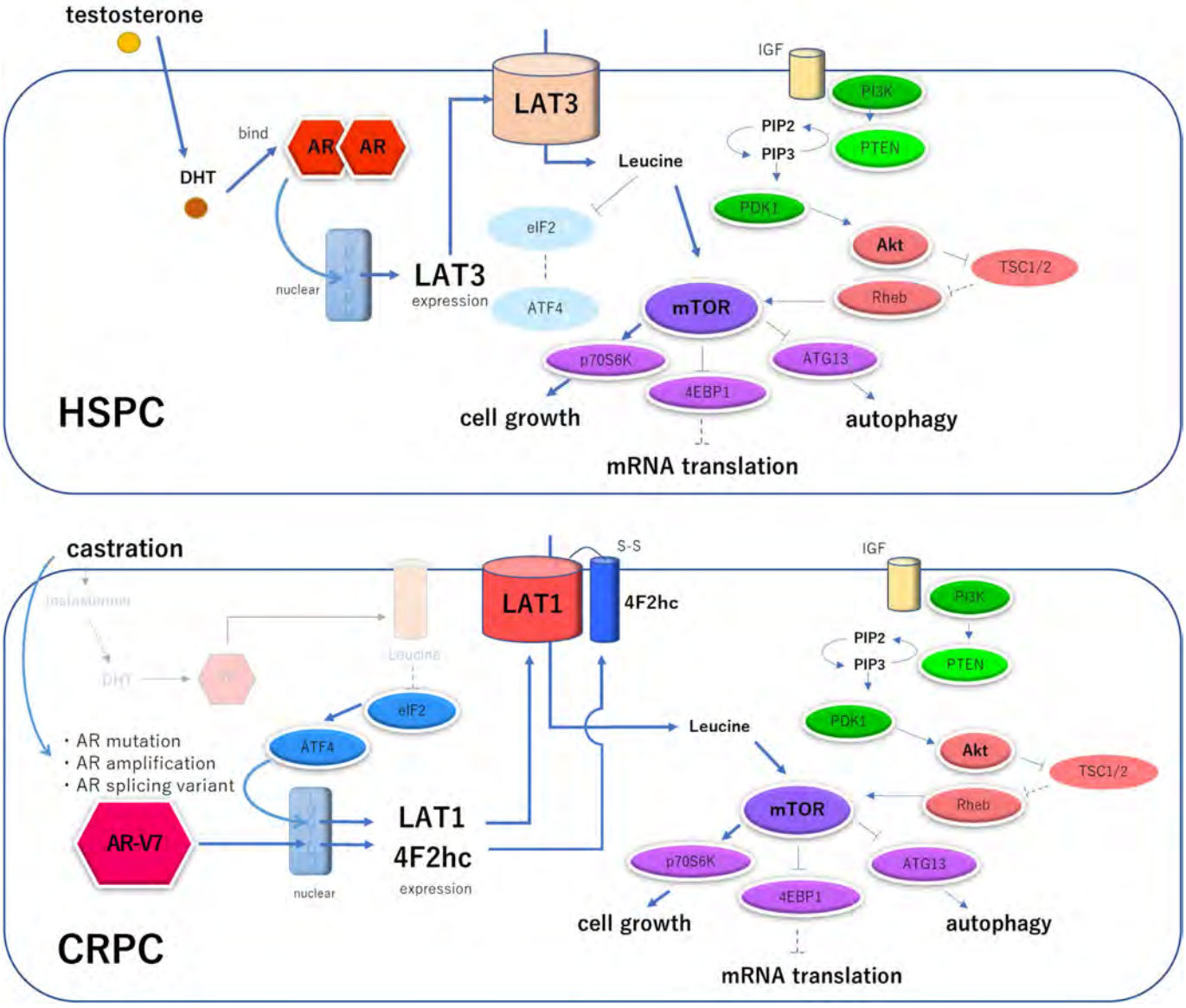

Fig. 4. Scheme shows the association of LATs and prostate cancer. The association of hormone-sensitive prostate cancer (HSPC) and castration-resistant prostate cancer (CRPC) with LAT is shown. In untreated HSPC, testosterone is metabolized by 5-alpha reductase to dihydrotestosterone (DHT), which binds to the androgen receptor (AR), forms a dimer, enters the nucleus, and drives LAT3 transcription, resulting in increased LAT3 expression, and contributes to the activation of the mTOR pathway. When hormone therapy is used as a treatment, testosterone disappears, i.e., castration occurs, and ARs that no longer bind DHT mutate, amplify, and form splicing variants. In particular, AR-V7 is able to enter the nucleus without testosterone stimulation, and 4F2hc is present in its downstream signaling. In addition, the depletion of leucine from the cells, which LAT3 took up, leads to the loss of eIF2 repression and the entry of ATF4 into the nucleus. It increases the expression of LAT1, and LAT1 and 4F2hc form a dimer, which allows leucine to enter the cell and promotes tumor cell growth. Reference:[68]

nephrectomy specimens and higher expression in the pT3-4 group compared to the pT1-2 group.[23]

Since the relationship between LAT1 and renal cancer remains unresolved, further analysis is warranted.

9. Conclusion

Although the relationship between malignancy and LAT1 has become clear in recent years, there are still many unanswered questions in urology. In addition, although there are a significant number of basic analyses, there are still few clinical reports.

The treatment of urological malignant tumors is in the midst of a transition from cell-killing anti-cancer drugs to the era of newer therapies represented by molecularly targeted drugs and immune checkpoint inhibitors. In prostate cancer, treatment options have expanded from classical hormone therapy and anti-cancer drugs to novel hormone drugs and PARP inhibitors that target *BRCA1/2* mutations.

LAT1 is involved in the growth and proliferation of malignant tumors at a completely different mechanism than conventional diagnostic and therapeutic targets. In other words, LAT1 is a useful target molecule for diagnostic imaging and therapy. Thus, LAT1 may cause a paradigm shift in cancer diagnosis and therapy.

Declaration of Competing Interest

The author has no conflict of interest to declare in this work.

Acknowledgements

This work was supported by [JSPS KAKENHI] grant number [21H03065, 21K19348,20H03813 and 20K09555].

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Review

Contribution of LAT1-4F2hc in Urological Cancers via Toll-like Receptor and Other Vital Pathways

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https://doi.org/10.3390/cancers14010229

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Review

Contribution of LAT1-4F2hc in Urological Cancers via Toll-like Receptor and Other Vital Pathways

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Simple Summary: LAT1-4F2hc complex is an important amino acid transporter. It mainly transports specific amino acids through the cell membrane, provides nutrition for cells, and participates in a variety of metabolic pathways. LAT1 plays a role in transporting essential amino acids including leucine, which regulates the mTOR signaling pathway. However, the importance of SLCs is still not well known in the field of urological cancer. Therefore, the purpose of this review is to report the role of the LAT1-4F2hc complex in urological cancers, as well as their clinical significance and application. Moreover, the inhibitor of LAT1-4F2hc complex is a promising direction as a targeted therapy to improve the treatment and prognosis of urological cancers.

Abstract: Tumor cells are known for their ability to proliferate. Nutrients are essential for rapidly growing tumor cells. In particular, essential amino acids are essential for tumor cell growth. Tumor cell growth nutrition requires the regulation of membrane transport proteins. Nutritional processes require amino acid uptake across the cell membrane. Leucine, one of the essential amino acids, has recently been found to be closely associated with cancer, which activate mTOR signaling pathway. The transport of leucine into cells requires an L-type amino acid transporter protein 1, LAT1 (SLC7A5), which requires the 4F2 cell surface antigen heavy chain (4F2hc, SLC3A2) to form a heterodimeric amino acid transporter protein complex. Recent evidence identified 4F2hc as a specific downstream target of the androgen receptor splice variant 7 (AR-V7). We stressed the importance of the LAT1-4F2hc complex as a diagnostic and therapeutic target in urological cancers in this review, which covered the recent achievements in research on the involvement of the LAT1-4F2hc complex in urinary system tumors. In addition, JPH203, which is a selective LAT1 inhibitor, has shown excellent inhibitory effects on the proliferation in a variety of tumor cells. The current phase I clinical trials of JPH203 in patients with biliary tract cancer have also achieved good results, which is the future research direction for LAT1 targeted therapy drugs.

Keywords: L-type amino acid transporter 1 (LAT1, SLC7A); 4F2 cell-surface antigen heavy chain (4F2hc, SLC3A2); urinary system tumors; diagnosis; targeted therapy

Citation: Zhao, X.; Sakamoto, S.; Maimaiti, M.; Anzai, N.; Ichikawa, T. Contribution of LAT1-4F2hc in Urological Cancers via Toll-like Receptor and Other Vital Pathways. *Cancers* **2022**, *14*, 229. https://doi.org/10.3390/cancers14010229

Academic Editor: David Wong

Received: 13 November 2021
Accepted: 2 January 2022
Published: 4 January 2022

1. Introduction

Continuous proliferative signaling is the main feature of malignant tumors [1]. These signals trigger tumor cells to divide, causing tumor cells to grow rapidly in an uncontrollable way. Among all of these nutrients, Eagle discovered in 1955 that essential amino acids (EAA) were required for cell growth in vitro [2]. Later, studies found that the uptake of EAA in malignant tumor cells was higher than in normal tissues [3–5]. After being delivered into

the cells, these amino acids were utilized to make proteins, nucleic acids, lipids, and ATP. Cancer cells have higher up-regulated transporters that facilitate the entrance of exogenous amino acids into cells, compared to normal cells, and the steady acquisition of amino acids by cancer cells is important for cancer growth [6]. HATs (heteromeric amino acid transporters) are a special type of solute transporter. They are made up of two subunits, one heavy and one light, that are linked by a conserved disulfide bond [7]. The heavy subunit is a member of the SLC3 family, whereas the light subunit belongs to the SLC7 family.

The SLC3 family now includes two glycoproteins (rBAT (SLC3A1)) and 4F2hc (SLC3A2, also known as CD98) [7]. Heavy subunits of the SLC3 family, such as 4F2hc, were discovered in 1998 and are necessary for the proper trafficking of the heterodimer to the plasma membrane [8].

Regarding the SLC7 family, Kanai first isolated a cDNA from rat C6 glioma cells through expression cloning in 1998. The cDNA encodes a new Na +-independent neutral amino acid transporter called LAT1 [9]. In 1999, Kanai's team further isolated a cDNA from the rat small intestine, which encodes another transporter called LAT2 [10]. The former two proteins belong to the solute carrier family 7 (SLC7). After that, LAT3 [11] and LAT4 [12] were gradually discovered. These two belong to the SLC43 family. The L-type amino acid transporter, which consists of all former four subunits (LAT1-4), is an important pathway for EAA to enter the cell. Subsequently, Wang found that (18) F-labeled fluoroalkyl phenylalanine derivatives as PET tracers were more likely to bind to LAT1 in tumors, and the specific accumulation of this tracer in tumor cells suggested that LAT1 was expressed in a large number of malignant tumors, thus preliminarily revealing the close relationship between LAT1 and malignant tumors [13]. In 2016, U.S. Food and Drug Administration approved trans-1-amino-3-18F-fluorocyclobutanecarboxylic-acid (anti-[18F]-FACBC) PET for the detection of prostate cancer in patients with elevated prostate-specific-antigen following curative treatment [14]. LAT1 is known to be the primary target of FACBC [15]. The usefulness of LAT1 in PET imaging has already been validated in clinical practice.

In a previous extensive review, Wang reported that among the four LAT transporters, LAT1 (SLC7A5) is overexpressed in various cancers, which is more widespread than the other three LAT transporters [3]. Subsequent research intensified and found that the complex composed of 4F2hc and LAT1 played play a key role in the occurrence and development of multiple human tumors. How to block the transport of nutrients by HATs to malignant tumor cells to achieve the purpose of inhibiting the occurrence and development of malignant tumor cells is an attractive research topic.

However, the importance of SLCs is still not well known in the field of urological cancer. In particular, LAT1 is a target of FACBC PET [15], which has important imaging implications in prostate cancer, following PSMA PET. Recently, 4F2hc, which binds to LAT1, has been identified as a specific downstream signal of AR-V7, a cause of castration resistance [16]. JPH203, a specific inhibitor of LAT1, has already completed Phase I clinical trials in Japan and may be applied to prostate cancer in the future [17].

Therefore, in this review, we summarized the latest advances in research on the role of the LAT1-4F2hc complex in urinary system tumors and emphasized the importance of the LAT1-4F2hc complex as a diagnostic and therapeutic target in urinary system tumors.

2. LAT1-4F2hc Complex and Structural Characteristics

LAT1 is made up of two layers of 12 putative transmembrane segments (TMs). TM1, TM3, TM6, TM8, and TM10 make up the inner layer, which is encircled by the outer layer. The outer layer is made up of TM2, TM4, TM5, TM7, TM9, TM11, and TM12. LAT1's N- and C-terminal ends are intracellularly localized, whereas 4F2hc's N- and C-terminal ends are intracellularly and extracellularly localized. The contact between 4F2hc and LAT1 is limited to one side of LAT1, while TM1 and TM6 of LAT1 are construction switches, which are essential for the alternate entry transport mechanism of the LeuT-fold transporters, and their positions are far away from the coordination of 4F2hc. Therefore, 4F2hc seems to stabilize the scaffold domain of LAT1 in the membrane, which may contribute to the

local conformational shift of gating elements (such as TM1, TM2, TM6, and TM10) during alternate entry cycles [18–20] (Figure 1A–C).

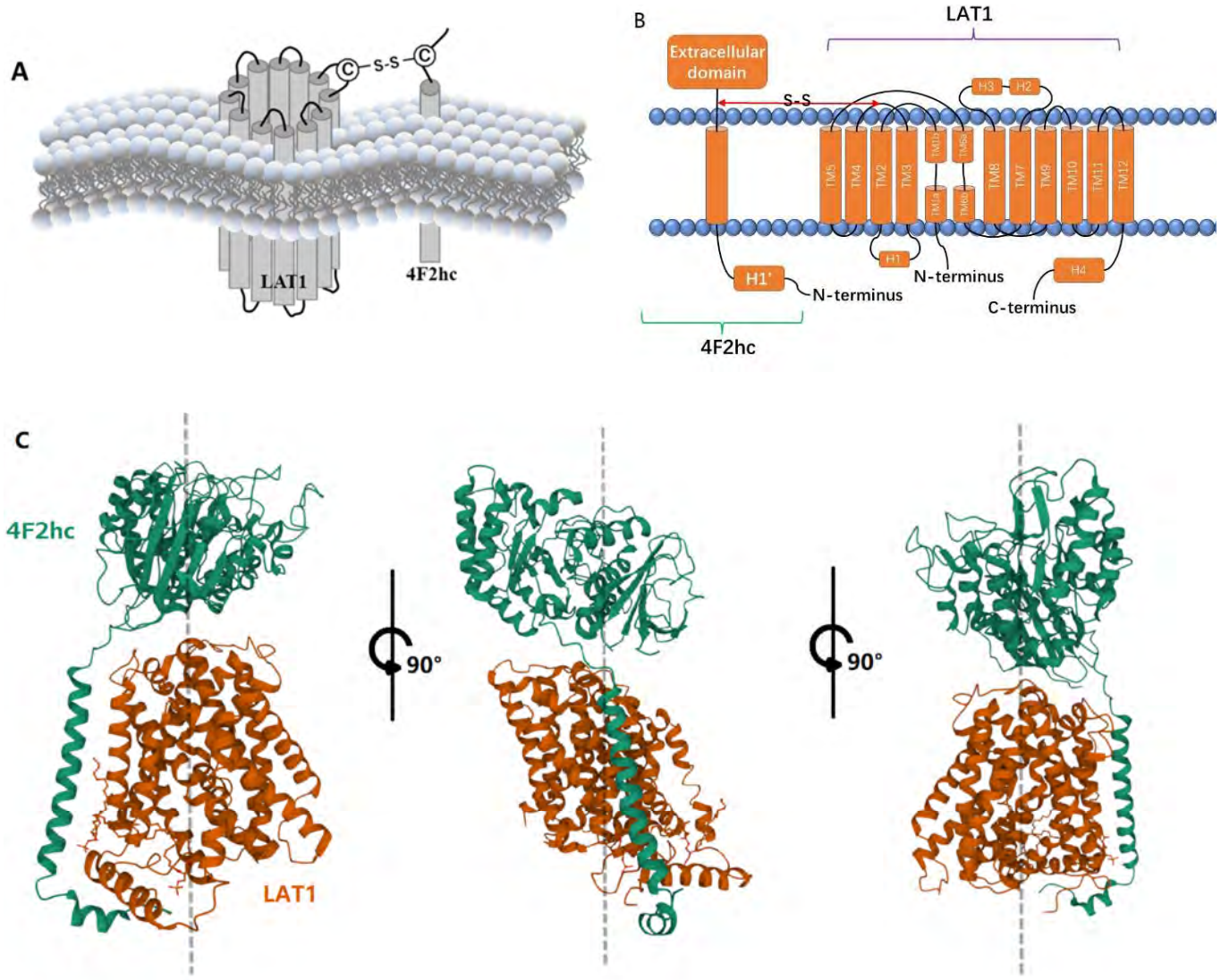

Figure 1. Structure of LAT1-4F2hc Complex: (**A**) Hypothetical model of the complex of LAT1 and 4F2hc; (**B**) LAT1 has 12 transmembrane units, while 4F2hc has only one. The two are covalently connected by disulfide bonds; (**C**) FIG1 (**C**) Images created using Mol*, the PDB ID: 6IRS, Structure of the human LAT1-4F2hc heteromeric amino acid transporter complex. [19], Mol* (D. Sehnal, S. Bittrich, M. Deshpande, R. Svobodová, K. Berka, V. Bazgier, S. Velankar, S.K. Burley, J. Koča, A.S. Rose (2021) Mol* Viewer: modern web app for 3D visualization and analysis of large biomolecular structures. Nucleic Acids Research. doi: 10.1093/nar/gkab314 [21]), and RCSB PDB.

According to the structure of LAT1-4F2hc heterodimeric amino acid transporter protein complex, 4F2hc had only one transmembrane helix that seemed to be unable to form a transmembrane transporter pore. It shows that 4F2hc has a lack of amino acid transport activity. In contrast, LAT1 shows a typical membrane transport protein helical bundle structure. That is the reason why past studies have reported that LAT1 is the only sole transport-competent unit, and 4F2hc does not play any significant role in the internal transport function [22]. Now, there are different views about it. Glycoprotein 4F2hc acts as a molecular chaperone to make LAT1 the final location on the cell membrane [23]. In the absence of 4F2hc, LAT1 is present in the intracellular compartment, while 4F2hc can independently reach the plasma membrane [8,23]. In the presence of LAT1, the surface

expression pattern of 4F2hc changes, restricting it to cell-cell adhesion sites [23]. 4F2hc is necessary for the transport of LAT1 to the plasma membrane, and LAT1 is believed to determine the transport properties of heterodimers. It is obvious that LAT1 and 4F2hc cannot work alone without each other. Meanwhile, in many forms of cancer, increased 4F2hc expression levels have been linked to a worse prognosis in several studies [24–27]. The most critical structure involved in the interaction of the complex is the disulfide bond between the two proteins [8,23]. The functional role of the disulfide bond is still unclear. It does not seem to be involved in the ectopic of the two proteins to the membrane, nor in the transport of amino acids. However, recent studies have shown that disulfide bonds are important for regulating 4F2hc-related cation channels [28].

LAT1-4F2hc heterodimeric amino acid transporter protein complex is a transmembrane transporter that independent of Na+ and pH. It imports large neutral amino acids (such as leucine and phenylalanine) for intracellular amino acid exchange (e.g., glutamine) [7,29], which are abundant in cells that require a constant supply of amino acids, such as nerve cells, activated T cells, placental cells, glial cells, and blood-brain barrier (BBB) endothelial cells [9,30,31]. In BBB, LAT1-4F2hc complex is stereospecific (L > D) [32]. Compared with LAT1 in peripheral tissues [33], it has a higher affinity for amino acids. Studies have shown that the affinity of LAT1 to intracellular amino acids is higher than that of extracellular amino acids, demonstrating that the quantity of intracellular substrate regulates LAT1 transport rate [34]. Due to its own transport characteristics, the LAT1-4F2hc complex often plays a key role in drug absorption, distribution and toxicity by mediating drug transmembrane transport, and often represents unexpected off-target of drugs [35].

3. LAT1/4F2hc and Human Diseases (Pain & Inflammation)

Existing studies have found that LAT1-4F2hc complex is widely associated with human diseases, such as inflammation, pain, hypoxia, and tumors [36–38].

Inhibition of LAT1 eliminated mTORC1 activation, plasmablast differentiation, and CpG (toll-like receptor TLR9 ligand)-stimulated B cell production of IgG and inflammatory cytokines. The influx of L-leucine through LAT1 regulates the activity of mTORC1 and the immune response of human B cells [37,38]. Among the most common nociceptive pathways, LAT1 may be a feasible new target for pain. LAT1 expression and regulation link it to key cell types and pathways related to pain. LAT1 regulates the Wnt/frizzled/β-catenin signal transduction pathway. The LAT1-4F2hc complex may also be involved in pain pathways related to T cells and B cells. The expression of LAT1 induces the activation of the mammalian target of rapamycin (mTOR) signal axis, which is related to inflammation and neuropathic pain. Similarly, hypoxia and tumors can induce the activation of hypoxia-inducible factor 2α, which not only promotes the expression of LAT1 but also promotes the activation of mTORC1 [36]. As the common node of the T cell, B cell, and mTOR pathway, LAT1-4F2hc plays a vital role in human diseases. It has also received increasing attention as an important target for autoimmune diseases, chronic pain diseases, and tumors.

4. LAT1/4F2hc and Tumors

Many tumor cells lines [39–41] and human malignancies, such as breast, prostate, lung, colorectal, and gliomas [42–47], have high levels of LAT1 expression. In these tumors, LAT1 plays an important role in growth and survival. RNA interference (RNAi) [44,48–51] and genetic disruption by zinc fingers nucleases-mediated [52] LAT1-knockout in cancer cells caused that leucine absorption and cell proliferation were both inhibited. As a result, LAT1 is being evaluated as a potential therapeutic target for reducing cancer cell growth and proliferation [53,54].

Similarly, in human neoplasms such as prostate cancer, gastric cancer, lung pleomorphic carcinoma, and neuroendocrine carcinoma, 4F2hc expression is upregulated [24,27,41,55]. Increased 4F2hc expression is linked to a worse chance of survival, cell proliferation, and metastasis [56]. Since 4F2hc binds with LAT1 on the membranous surface of cancer cells, these results are not difficult to understand.

The LAT1-4F2hc complex is also closely related to tumor glutamine metabolism. The amount of glutamine required by cancer cells exceeds the supply produced by endogenous synthesis, resulting in the up-regulation of glutamine metabolism in many carcinogenic changes. LAT1-4F2hc complex controls the flux of glutamine and other amino acids involved in glutaminolysis and glutamine-regulated homeostasis [35]. LAT1-4F2hc complex exchanges Gln for leucine and other amino acids, which can lead to mTOR activation.

By influencing the mammalian target protein of rapamycin complex 1 (mTORC1), the amino acid leucine has been demonstrated to increase protein synthesis and accelerate cell development, whereas LAT1 has been linked to mTORC1 signaling and, as a result, cancer progression [6,57].

In cancer cells, however, LAT1 not only boosts mTORC1 activity but also enhances MYC and EZH2 signaling. Through the AKT, MAPK, and cell-cycle related P21 and P27 signal pathways, 4F2hc has been demonstrated to affect cancer cell proliferation. The expression of 4F2hc and LAT1 is reportedly codependent, and the downregulation of either subunit destabilizes the partner [8]. (Figure 2, Table 1).

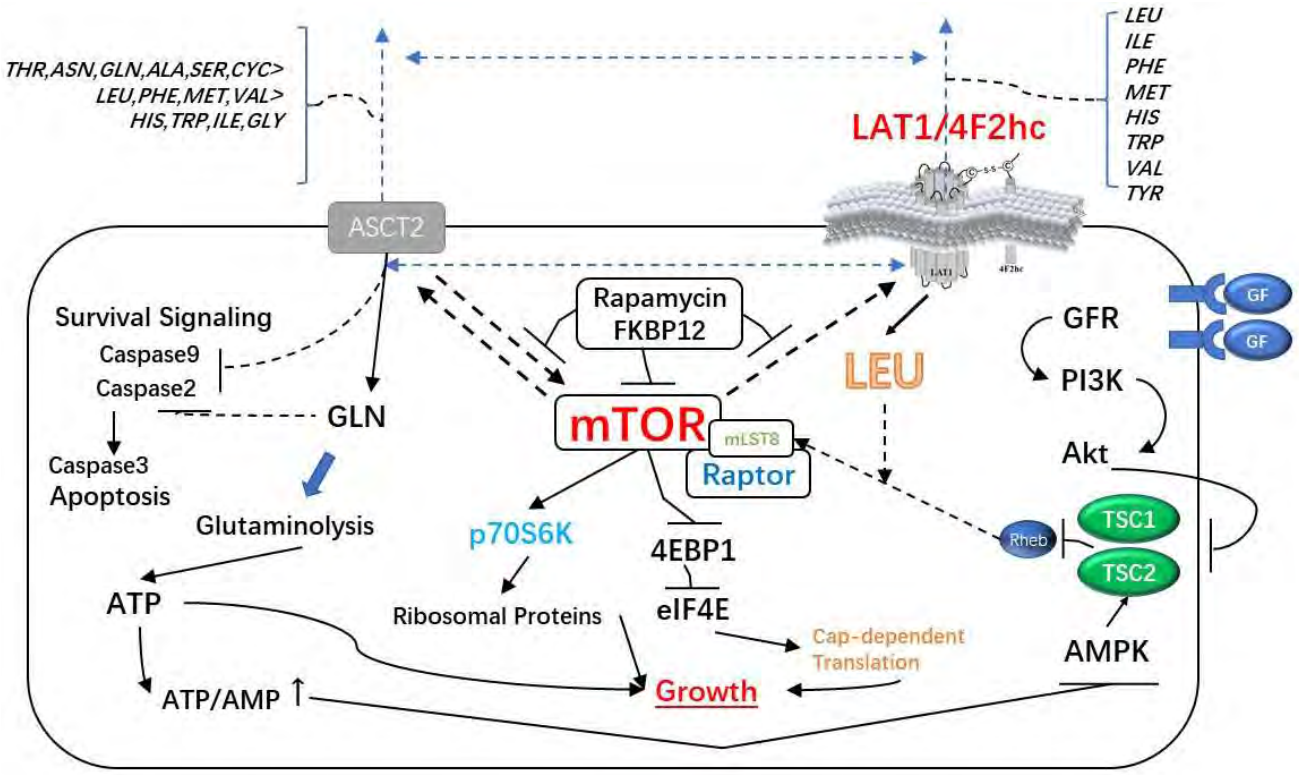

Figure 2. The Major Signaling Pathways Affected by LAT1-4F2hc Complex: The LAT1-4F2HC complex not only enhances mTORC1 activity but also enhances MYC and EZH2 signaling pathways. Moreover, it can affect the proliferation of cancer cells through AKT, MAPK and cell cycle-related P21 and P27 signaling pathways.

Table 1. LAT1-4F2hc and Common Tumors.

	Cancer Types	Cell Lines	Downstream Effects of LAT1/4F2hc	Other Related Factors	References
LAT1-4F2hc Complex	NSCLC	A549, H1299	Mice with smaller tumors, lower leucine absorption, lower mTORC1 activity, amino acid stress, lower proliferation, and lower EZH2 expression and activity	Ki-67, VEGF, CD31, CD34, HIF-1a, mTOR, ASCT2	[27,52,58–63]
	Gastric cancer	SGC-7901, MKN-45, MGC-803, CRL-5974	Deceases in proliferation, migration and invasion	Ki-67	[25,64–69]
	Pancreatic cancer	MIA, Paca-2	Reductions in mTORC1 activity, decreases in proliferation and angiogenesis	Ki-67, VEGF, c-Myc, CD147	[50,70–74]
	Biliary tract cancer	KKU-M055, KKU-M213	JPH203 first in human phase I clinical trial. Well-tolerated.	Ki-67	[75–81]
	Ovarian cancer	SKOV3, IGROV1, A2780, OVCAR-3	Decreases in proliferation	ASCT2, SN2, p70S6K, LAT2	[82–85]
	Breast cancer & TNBC	MCF-7, ZR-75, MDA-MB-232	Decreases in proliferation	ADS, HER2, TN, Ki-67, ER, PgR	[45,86–90]

5. LAT1/4F2hc and Urological Tumors

5.1. LAT1/4F2hc and Prostate Cancer

LAT1-4F2hc complex plays an important role in growth and survival in PCa cells. Sakata used LAT1 as a biomarker for highly malignant prostate cancer in 2009 [47]. The increased expression of LAT1 in prostate cancer is a new independent biomarker of high malignancy that can be used to estimate prognosis in conjunction with the Gleason score [47].

Trans-1-amino-3-18F-fluorocyclobutanecarboxylic-acid (anti-[18F]-FACBC) is an amino acid PET tracer, which shows good prospects in visualizing PCa [91]. The tracer is used for the evaluation of l-amino acid transport, LAT1 is known to be the primary target of FACBC [15]. In 2016, 18F-FACBC has been approved by the US Food and Drug Administration (FDA) and the European Commission (EC) to detect PCa in patients with elevated PSA after previous treatments [14]. Approval is based on encouraging diagnostic performance and histologically confirmed data from patients with biochemical relapse [92]. Recently, it was included in the National Comprehensive Cancer National (NCCN) guidelines for the treatment of patients with recurrent PCa. The usefulness of LAT1 in PET imaging has already been validated in clinical practice.

Wang reported [57] that when LAT activity was inhibited, activating transcription factor 4–mediated overexpression of amino acid transporters such as ASCT1, ASCT2, and 4F2hc occurred, all of which were regulated by the androgen receptor. LAT suppression inhibited M-phase cell cycle genes regulated by E2F family transcription factors, including UBE2C, CDC20, and CDK1, which are important castration-resistant prostate cancer regulators. In silico analysis of BCH-downregulated genes revealed that in metastatic castration-resistant prostate cancer, 90.9 percent are statistically significantly upregulated. Finally, in vivo, LAT1 knockdown decreased tumor development, cell cycle progression, and spontaneous metastasis in xenografts [57].

Patel studied the functional characterization and molecular expression of large neutral amino acids of LAT1 in prostate cancer PC-3 cells [93]. It proves that LAT1 is mainly responsible for the uptake of large neutral amino acids and has functional activity in PC-3

cells. The fact that Ile-quinidine generates a considerable increase in absorption compared to quinidine suggests that LAT1 could be used to improve the cellular permeability of poorly cell-permeable anticancer medicines. This cell line can also be utilized as an in vitro model to investigate the interaction of large-scale neutral amino acid conjugated pharmaceuticals with the LAT1 transporter [94].

In PCa cell lines, DU145 cells had the highest levels of 4F2hc protein expression, followed by PC-3 and C4-2 cells. In C4-2 and DU145 cells, 4F2hc expression was found to be substantially greater than LAT1 expression. Cell growth, migration, and invasion are all inhibited by Si4F2hc. 4F2hc and LAT1 expression in PCa tissue and association with clinical variables. The expression levels (4F2hc and LAT1/high and low) are associated with various tumor prognoses [24]. The data from the same study [24] revealed that SKP-2 is a downstream and particular target gene of 4F2hc. SKP-2 is associated with cell cycle, DNA replication, and cell division.

5.1.1. AR and LAT1-4F2hc Complex in CRPC (AR/AR-V7 and 4F2hc Promotes the Development of CRPC)

Xu reported that the up-regulation of LAT1 during anti-androgen therapy promotes the progression of PCa cells [44]. In hormone-resistant prostate cancer cell lines, LAT1 was shown to be substantially expressed. Knocking down LAT1 in LNCaP and C4-2 cells can drastically reduce cell proliferation, migration, and invasion. In patients receiving androgen deprivation therapy, high LAT1 expression was linked to a significantly shorter prostate-specific antigen recurrence-free survival [44].

Another study demonstrated a potential relationship between AR-V7 and 4F2hc [16]. AR-V7 activates downstream target genes in the absence of androgens. 4F2hc (SLC3A2) is one of the downstream target genes of AR-V7. AR-V7 gene knockdown leads to a decrease in the level of H3K27ac at the 4F2hc locus. The decrease in the expression of 4F2hc indicates that AR-V7 has a certain effect on the activation of 4F2hc expression. In clinical samples, the expression level of 4F2hc in benign lesions and primary PCa tissues was low, while the expression level of 4F2hc in CRPC tissues was significantly increased. The expression of 4F2hc in PCa patients with high AR-V7 expression is higher than that in PCa patients with low AR-V7 expression.

When LNCaP and LNCaP95 cell lines were treated with siRNA against 4F2hc, cellular growth was significantly suppressed [16]. Down-regulation of 4F2hc inhibited cell proliferation through apoptosis and cell senescence [16].

5.1.2. LAT1/4F2hc Expression Is Coordinately Regulated during Prostate Cancer Progression (HSPC to CRPC)

Not all prostate tumor cell lines are closely related to LAT1. Otsuki found that LNCaP cells mainly express LAT3, and LAT1 was primarily expressed in DU145 and PC-3 cells [95]. Xu's research also gave similar results [44]. LAT3 was abundantly expressed in AR-expressing LNCaP and C4-2 cells, whereas it was barely expressed in AR-negative PC3 and DU145 cells, according to Rii's study [96].

Wang [97] reported the fact that LAT1 is highly expressed in androgen-insensitive PC-3 cells but LAT3 is highly expressed in androgen-sensitive LNCaP cells could be explained by transcriptional regulation of LAT1 and LAT3 expression. Changes in the microenvironment, such as starvation or hormone deprivation, can promote cancer formation and alter LAT1 and LAT3 expression. Reduced androgen receptor signaling may result in decreased LAT3 expression and, as another result, higher LAT1 expression. The results were confirmed by both nude mice samples and human samples [97]. LAT3 expression was higher in amplified AR patients. In a dose-dependent way, DHT stimulation enhanced LAT3 expression. Bicalutamide inhibited the effect of DHT on LAT3 expression. DHT treatment significantly boosted AR expression, which was reduced by bicalutamide [96] (Figure 3).

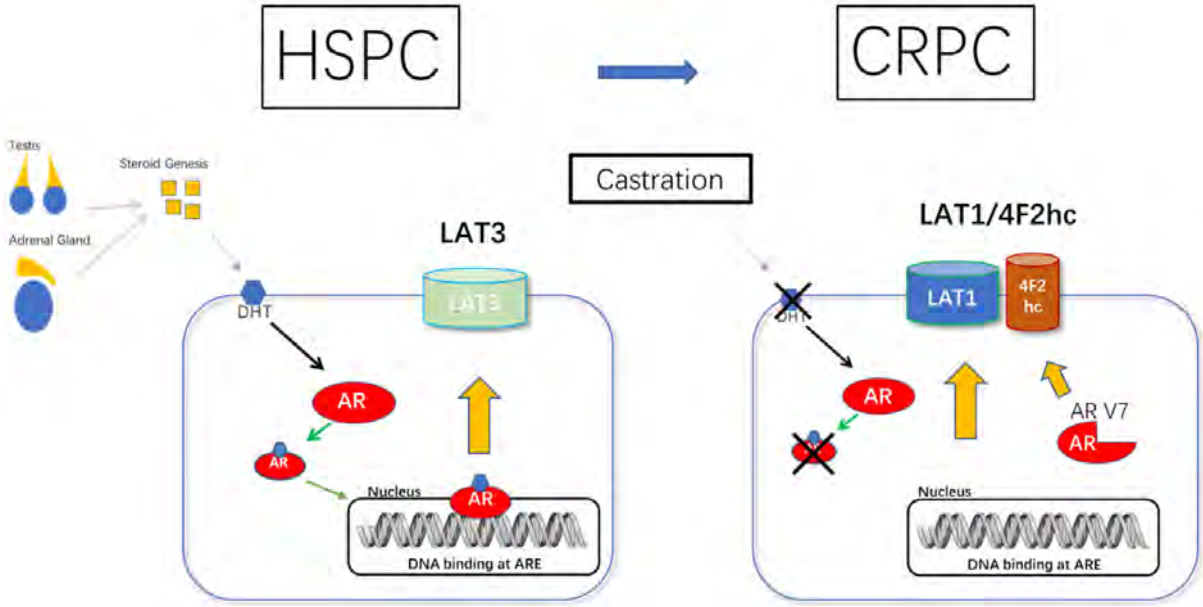

Figure 3. LAT Expression is Coordinately Regulated During Prostate Cancer Progression: Proposed model of LAT1-4F2hc/LAT3 in HSPC to CRPC. As HSPC progresses to CRPC, AR acts in reverse to cause low expression of LAT3 and high expression of LAT1.

Since high AR-V7 expression is one of the most common features of CRPC, AR-V7 expression following LH-RH therapy up-regulates the 4F2hc expression [16].

Based on the above evidence, LAT1/4F2hc can be independent PCa biomarkers and therapeutic targets, respectively. They can also collectively influence the transformation of PCa to CRPC and promote both progressions through the mentioned pathways below (Figure 4).

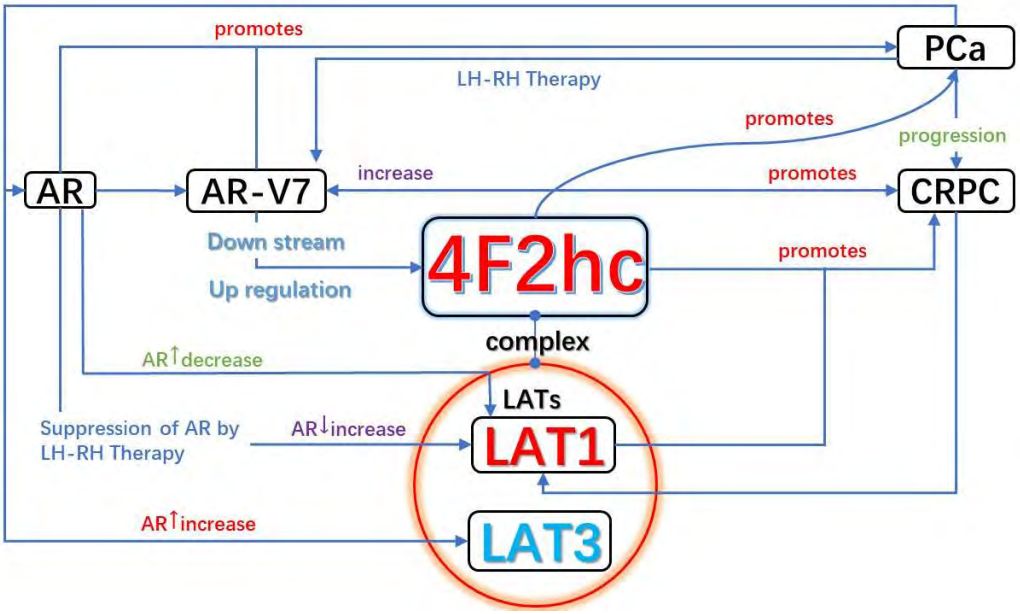

Figure 4. Relationship of LAT1-4F2hc and PCa & CRPC: The relationship between LATx-4F2hc and AR(AR-V7) and different stages of prostate cancer. Reduced androgen receptor signaling and variation of androgen receptors may result in decreased LAT3 expression and higher LAT1 expression.

5.2. LAT1/4F2hc and Renal Cancer

There are few studies on LAT1 and renal clear cell carcinoma. In 2013, Hironori [42] studied the expression of LAT1, LAT2, LAT3, LAT4, and 4F2hc mRNA in clear cell renal cell carcinoma tissues. It was found that the expression of LAT1 mRNA in tumor tissue was considerably higher than in non-tumor tissue, but the expression of LAT2 and LAT3 mRNA was lower. There was no difference in LAT4 and 4F2hc mRNA expression between tumor and non-tumor tissues. Poorly differentiated tumors, local invasion, microvascular invasion, and metastasis are all linked to increased LAT1 mRNA expression. Higher LAT1 mRNA levels in the blood are linked to a shorter total survival period. Phosphorylated S6 ribosomal protein levels are related to metastatic potential. The level of phosphorylated S6 ribosomal protein is positively linked with the expression of LAT1 mRNA in primary cancers [42].

Higuchi investigated the LAT1 expression profile in RCC tissues as well as its relationships with clinical variables retrospectively [98]. Most of the tissues (92 percent) had cancer-associated LAT1 expression. Patients with high LAT1 expression levels had lower overall survival and progression-free survival than those with low LAT1 expression levels, and these correlations were confirmed by univariate and multivariate analyses [98].

Tumors grow and evolve through continuous crosstalk with the surrounding microenvironment. New evidence shows that angiogenesis and immunosuppression often occur simultaneously to deal with this crosstalk [99]. At present, one strategy to achieve a higher clinical response in the study of renal cell carcinoma is to produce a more effective anti-tumor contraction by combining multiple immune checkpoints. However, the toxicity profile is higher [100]. T cells can shape tumor blood vessels and tumor endothelial cells, prevent the recruitment and infiltration of effector immune cells while remodeling ECM, and further inhibit the migration and infiltration of functional immune cells. The tumor vascular system actively participates in immunosuppression. The abnormal pathophysiological mechanism of tumor vessels can lead to the production of immunosuppressive molecules and inhibit the function of effective T cytotoxic cells. At the same time, the production of chemokines and cytokines promotes the differentiation and activation of immunosuppressive cells. These cells can also inhibit the activity of cytotoxic T cells. On the contrary, in the blood vessels, these mechanisms also down-regulate a variety of adhesion molecules, which are very important for the rolling, adhesion, and transport of T cells into the cancer environment. The normal tumor vascular system can improve T cell infiltration, enhance immune response, stop the immunosuppressive environment, make it a more immunoactivated phenotype, and work together with cancer immunotherapy. Anti-vascular endothelial growth factor receptor (anti-VEGFR) is the first to realize the normalization and functional recovery of tumor vascular system by tissue perfusion and reducing intratumoral hypoxia [99]. In the current studies of cancers [71,101,102], angiogenesis in vitro/in vivo experiments was inhibited by eliminating the function or expression of LAT1. It regulates proliferation, translation, and angiogenesis VEGF-A signal [102]. LAT1 is a central transporter of essential amino acids in human umbilical vein endothelial cells [103]. LAT1 also mediated miR-126 on primary human lung microvascular endothelial cells' angiogenesis via regulation of mTOR signaling [104]. LAT1 expression correlated significantly with CD98, VEGF, CD34 expression, and microvessel density in the primary and metastatic sites of tumors [41,81,105–108]. VEGF and CD34 are also related to angiogenesis. These studies further revealed the dual role of LAT1-4F2hc in tumor cells and stromal endothelial cells. The therapeutic inhibition of LAT1-4F2hc may provide an ideal choice for strengthening anti-angiogenesis therapy. Lat1-4f2hc is a potential therapeutic target for anti-tumor angiogenesis and maintenance of the normal vascular system. Therefore, the combination of antiangiogenic therapy and immunotherapy seems to have the potential to break the balance of the tumor microenvironment and improve the treatment response of renal cell carcinoma. It can be a novel paradigm to envision tailored approaches in renal cell-carcinoma and other urological tumors.

5.3. LAT1/4F2hc and Bladder Cancer

In 2002, Kyung reported the characterization of the system L amino acid transporter in T24 cells [93]. T24 human bladder cancer cells express LAT1 and its associated protein 4F2hc in the plasma membrane, however, T24 cells do not express the other system L isoform LAT2. The majority of [14C]L-leucine uptake is mediated by LAT1 in T24 cells [93].

Baniasadi [109] reported the gene expression profile of inhibiting LAT1 in T24 human bladder cancer cells. BCH influences the expression of a vast number of genes involved in cell survival and physiological function, according to researchers. These findings contribute to a better understanding of the intracellular signaling pathways involved in cell growth suppression produced by LAT1 inhibitors, which could be utilized as a target for anticancer drug development [44,109].

Maimaiti studied the expression profile and functional role of LAT1 in bladder cancer [110]. This is the first study to show that LAT1 plays a role in bladder cancer, and it also found IGFBP-5 to be a new downstream target for inhibiting LAT1. High LAT1 expression was found to be an independent predictive factor for overall survival in multivariate analysis. Patients with high LAT1 and IGFBP-5 expression had a significantly lower overall survival than those with low expression. LAT1 levels are linked to pathological alt staging, LDH, and NLR. In vitro, inhibiting LAT1 prevents cell proliferation, migration, and invasion. In aggressive BC patients, IGFBP-5 expression is also linked to a better prognosis [110] (Table 2).

Table 2. LAT1-4F2hc and Urological Tumors.

LAT1-4F2hc and Urological Tumors										
Cancer Types	**Cell Lines**	**Expression**		**Inhibitors**		**Be Inhibited Downstream Effects**		**Other Related Factors**	**Meanings**	**References**
		LAT1	**4F2hc**	**LAT1**	**4F2hc**	**LAT1**	**4F2hc**			
Prostate cancer	LNCAP	↑(Not express [95])	↑	BCH, JPH203, R1881, ESK242	BCH, JPH203, R1881, AR-V7 knockdown	Lower leucine absorption, Lower mTORC1 activity, Amino acid stress, Down regulation of ATF4-mediated genes, Reduced tumor metastasis ability in PC3-CRPC metastatic tumor mouse model.	Lower proliferation, higher apoptosis, and several gene expression changes.	LAT3, ATF4, ASCT1, ASCT2, SKP-2, ADT (LH-RH Therapy), y+LAT2, mTORC1, Ki-67, AR, AR-V7, SLFN5	A biomarker of PCa. Associated with high Gleason score, improving drug delivery in PCa cells. Specific antibodies to LAT1 can inhibit tumor growth. Expression changes when hormone ablation and in metastatic lesions. The expression levels of LAT1 and 4F2hc suggest different prognosis respectively.	[16,24,44,57,94,95,97,111]
	LNCAP95	↑	↑							
	C4-2	↑	↑							
	PC3	↑	↑							
	DU145	↑	N/A							
	VCAP	↑	↑							
Renal cancer	Caki-1	↑	N/A	JPH203	JPH203	Lower mTORC1 activity, Reduced p70S6K and 4E-BP1.	N/A	S6 ribosomal protein (Ser-235/236)	An RCC biomarker for diagnosis and treatment. Related to the poorer differentiation, associated with local invasion and microscopic vascular invasion. LAT1-mRNA is a target for therapy. Promising prognostic markers. High LAT1 expression suggests a poor prognosis (OS & PFS).	[42,98]
	ACHN	↑	N/A							
	ccRCC tissue	↑	→(by mRNA detection)							
Bladder cancer	T24	↑	↑	BCH, JPH203, SiLAT1	BCH	Cell growth inhibition, inhibit phosphorylation of MAPK/Erk, AKT, p70S6K, and 4EBP-1. Decreases in migration and invasion activities.	Reduced Leucine intake and tumor cell growth.	P27, Ki-67, IGFBP-5	An independent prognostic factor. Associated with the tumor stage.	[93,109,110,112]
	5637	↑	N/A							

6. Inhibitors of LAT1/4F2hc and Targeted Therapy

Due to its own transport characteristics of the SLC family, the LAT1-4F2hc complex often plays a key role in drug absorption, distribution and toxicity by mediating drug transmembrane transport [35]. However, only a small number of SLCs have been locked by drugs or chemical probes till now. Three main factors hinder the development of new chemical entities that can regulate SLC activity. First, most studies on this super population are relatively insufficient, and the biological functions or substrates of many SLCs are still unclear. Second, there is a lack of high-quality biological tools, specific, and reliable reagents and special databases. Finally, the number of functional analyses required to study such diverse objectives is still limited [113]. It is reported radioligand uptake assays have been widely employed to study LAT1 [114], but the radioligand uptake assays cannot distinguish inhibitors from substrates. The LAT1-4F2hc complex is overexpressed in many cancer cells and is thought to be a viable anticancer therapeutic target since inhibiting it reduces cancer cell viability dramatically.

BCH and JPH203 are LAT1-4F2hc complex inhibitors that have been studied extensively. BCH is a non-metabolic leucine analogue. In 2006, Baniasadi [109] found that BCH has an impact on the expression of many genes involved in cell survival and physiological activity. These data help to understand the intracellular signal transduction of cell growth inhibition induced by LAT1 inhibitors and can be used as a candidate for anticancer drug therapy [109]. Later studies proposed the use of N-butyl-N- (4-hydroxybutyl) nitrosamine (BBN) treatment to induce high expression of LAT1/4F2hc in rat bladder cancer cells [101] and proposed some directions for anti-LAT1/4F2hc drugs. JPH203 was discovered by Oda in 2010 and was originally known as KYT-0353 [115]. JHP203 is a highly selective LAT1 inhibitor produced by synthetic chemistry and in vitro screening based on triiodothyronine (T3). JPH203 showed excellent selective inhibition of LAT1 and showed potential as a novel antitumor agent. JPH203 interferes with constitutive activation of mTORC1 and Akt, reduces c-MyC expression, and triggers a folding protein response mediated by CHOP transcription factors associated with cell death [116]. Since then, several studies have confirmed that JPH203 has an impressive inhibitory effect on the growth of common tumor cells, such as colon cancer [115,117], gastric carcinoma [64], medulloblastoma [118], osteosarcoma [119], thyroid cancer [120,121], endocrine-resistant breast cancer [122], pituitary tumor [123], head and neck cancer cells [124], and T-cell Acute lymphoblastic leukemia (T-ALL)/lymphoma (T-LL) cells [116], etc.

In terms of urinary tumors, Maimaiti [110] found that in bladder cancer cells JPH203 inhibits the absorption of leucine by >90%. JPH203 inhibits the phosphorylation of MAPK/Erk, AKT, p70S6K, and 4EBP-1. JPH203 inhibits IGF-mediated igfb5 expression and AKT phosphorylation [110].

In the area of RCC, Higuchi [98] has tested the effects of JPH203 on RCC-derived Caki-1 and ACHN cells. JPH203 suppressed the proliferation of various cell types in a dose-dependent manner. According to the findings, the migration and invasion operations were stifled by JPH203 [98].

In the area of PCa, Otsuki [95] found that LAT1 was primarily expressed in DU145 and PC-3 cells. BCH or JPH203 inhibited leucine uptake and cell proliferation in a dose-dependent manner [95]. A Phase I clinical study found that JPH203 was well-tolerated and provided promising activity against biliary tract cancer [17]. The authors are currently planning Phase I and II study of JPH203 in CRPC [17].

These studies also show the potential of JPH203 for the treatment of urological cancers.

In 2021, Yan [125] synthesized three LAT1 inhibitors, JX-075, JX-078, and JX-119, and used cryo-EM to solve the inhibitors' complex structures with the LAT1-4F2hc complex. They also solved the LAT1-4F2hc complex coupled with Diiodo-Tyr's cryo-EM structure. LAT1 is found in an outward-occluded conformation in all the combinations of these complexes. These structures might reflect two distinct inhibitory processes, giving significant information for medication development in the future [125].

Of particular interest is the first Phase I clinical trial of JPH203 [17]. Although several studies have demonstrated that JPH203 can inhibit leucine uptake by tumor cells and show concentration-dependent cytotoxicity in vitro or good results in transplanted tumor models, Phase I clinical trial in humans is a milestone. Okano assessed dose-limiting toxicity in the first cycle using the 3 + 3 design. Seventeen Japanese patients with advanced solid tumors were enrolled and treated daily with JPH203 intravenously for 7 days. The maximum safe tolerated dose of JPH203 was defined as 60 mg/m^2. The suitable RP2D is 25 mg/m^2. Partial response was observed in one biliary tract cancer (BTC) patient at 12 mg/m^2, and disease control was achieved in three of the six BTC patients at both the 12 mg/m^2 and 25 mg/m^2 levels. The disease control rate of BTC was 60%. The JPH203 molecule is predominantly metabolized into Nac-JPH203 by N-acetyltransferase 2 in liver cells [126]. Patients' N-acetyltransferase 2 phenotype (rapid/non-rapid) was found to predict the safety and efficacy of JPH203. A lower Nac-JPH203/JPH203 ratio is critical for maximizing the anti-tumor effect of JPH203 [17].

Of course, there are still some deficiencies and limitations in the study of urinary tumors and LAT1-4F2hc complexes mentioned above.

In BBN-induced bladder cancer, LAT1-4F2hc was not expressed by porous endothelial cells. Whether LAT1-4F2hc expression depends on endothelial cell structure is unclear. Fenestration of microvascular endothelial cells is not a stable event, because endothelial cells with fenestration in BBN-induced rat bladder cancer were transformed into endothelial cells without fenestration 5 min after injection of VEGF inhibitor, and fenestration recovered 30 min later [101]. The molecular mechanisms of amino acid transport in normal and tumor microvascular endothelial cells need further study. However, the LAT1-4F2hc complex is closely related to angiogenesis [41,71,81,101,102,105–108]. This makes it possible for the LAT1-4F2hc complex to improve the effectiveness of cancer immunotherapy by improving immune vascular crosstalk [99].

In prostate cancer-related experiments, although downregulation of LAT1 and LAT3 in tumor cells inhibits the growth of prostate cancer cells, it remains to be determined what other mechanisms of prostate cancer resistance can be triggered by targeting LAT1 (such as activation of ATF4).

Most of the studies were conducted in vitro, not in vivo. Although the phase I clinical trial of JPH203 against biliary tract cancer has achieved good results, the clinical trial has not yet involved any urinary tumors. In addition, the number of patients included in some studies is relatively small, or the follow-up time is not long, and the prognostic impact of LAT1 inhibition on tumor patients with different stages has not been thoroughly solved. Most of the specimens studied are in vitro tumor specimens after surgery, and the expression of LAT1-4F2hc in early tumors and its influence on tumors are also a key link that needs to be studied.

Finally, targeted therapy of LAT1-4F2hc does not directly kill cancer cells, but blocks amino acid transport, resulting in loss of nutritional basis and self-apoptosis of cancer cells. This has led some investigators to suggest that targeting LAT1-4F2hc is more suitable for slow-progressing tumors. Therefore, further studies are needed to obtain more evidence that LAT1-4F2HC therapy is also suitable for highly aggressive and rapidly progressing tumors.

7. Conclusions

Significant Contribution of the LAT1-4F2hc in Urological Cancers

These studies and experiments above are helping us to understand how cancer cells metabolize differently from normal cells, as well as the therapeutic targets that could be interfered with in these different metabolisms of proliferation. The abnormal proliferation of tumor cells usually depends on the nutrient microenvironment generated by these abnormal metabolic patterns. Recognizing and blocking the nutrient absorption pathways of malignant tumors are usually the key points in the diagnosis and treatment

of malignant tumors. The LAT1-4F2hc complex is such a target with both diagnostic and therapeutic significance.

The LAT1-4F2hc complex mediates a variety of pathways, such as T cells, B cells, and mTOR pathways, and is also closely related to Toll-like receptors and vascular endothelial growth factors. This has caused the LAT1-4F2hc complex to become a common factor in many diseases, such as autoimmune diseases, pain, tumors.

The clinical significance of the LAT1-4F2hc complex in urinary cancer has gradually begun to be explored and confirmed, just like in other tumor cells. LAT1-4F2hc upregulation seems to be a common phenomenon in cancers. It is a reliable tumor biomarker and the target of imaging tracer, which can be used for the diagnosis and prognosis of urinary malignant tumors. It is also a meaningful therapeutic target. In fact, great efforts have been made to decipher the biology of LAT1-4F2hc. While a complete scenario has not yet been painted, a combination of bioinformatics, in vitro, and animal experiments has revealed some previously unknown aspects of LAT1-4F2hc transport mechanisms, substrate specificity, and regulation. These results provide a strong basis for pharmacological studies in which inhibitors of LAT1-4F2hc, such as JPH203. JPH203 can act well on a variety of tumor cells. Its phase I clinical trial in humans is a great milestone for researchers and patients.

However, the study of LAT1-4F2hc is still rare and not thorough in the field of urinary tumors. The results obtained so far are not fully in line with the fact that LAT1-4F2hc should play a prominent role in the field of urinary cancers. Its transport, regulation of expression/function, effects of posttranslational modifications on its stability/activity, interactions with other amino acid transporters and upstream and downstream genes, reaction with chemotherapy sensitivity/resistance, relationship with immunotherapy of sensitivity/resistance, is worthy for further research. In addition, Whether the phase I or II clinical trials of JPH203 in patients with urinary tumors can improve the prognosis of urinary tumors and whether there are corresponding biomarkers that can be used to predict the sensitivity and prognosis of inhibitors are also worthy of study.

As a future direction, we are currently pursuing the utility of LAT1 as a biomarker in urological tumors. In recent years, the usefulness of liquid biopsy has been suggested in clinical practice. The expression of LAT1 in blood, including CTCs, ctDNA, and Exosome, is currently being examined through collaborative research.

We hope to prove its usefulness not only as an inhibitor but also as a companion di-agnostic agent in the near future.

In conclusion, LAT1-4F2hc plays an important role in the diagnosis, treatment, and prognosis assessment of urinary system tumors. Cancer-related amino acid transporters may change the diagnostic and treatment strategy of urological tumors in near future.

Author Contributions: X.Z. contributed to collecting bibliography, preparing figures, and writing; S.S. and M.M. contributed to drawing structure figures and writing; S.S., N.A. and T.I. contributed to supervision of all the activities. The first draft of the manuscript was prepared by X.Z., M.M. performed subsequent amendments. S.S. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The present study was supported by grants from the Japan Society for the Promotion of Science (20H03813 to T.I., 20K09555 to S.S., and 21H03065 to N.A.) and Japan China Sasakawa Medical Fellowship (to X.Z.).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Chapter 3

How Serum Testosterone Levels Determine the Treatment Strategy of Advanced Prostate Cancer

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Abstract

Most men with metastatic prostate cancer who receive androgen deprivation therapy (ADT) eventually became castration-resistant prostate cancer (CRPC) patients. In this review, we describe the role of serum testosterone (TST) levels in the progression and prognosis of prostate cancer based on several clinical studies of prostate cancer, and how to use testosterone levels to achieve the best treatment effect in different stages of the course.

Our data suggested both nadir testosterone < 20 ng/dL and testosterone reduction ≥ 480 ng/dL to be key prognostic factors for

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In: Horizons in Cancer Research. Volume 85
Editor: Hiroto S. Watanabe
ISBN: 979-8-88697-698-4

primary androgen deprivation therapy (ADT) in advanced prostate cancer. Serum TST 13 ng/dL is the dividing point that determines the response and efficacy of CRPC to drug therapy. Patients with serum TST > 13 ng/dL had better curative effects on novel androgen receptor (AR) antagonist medicines. However, those serum TST < 13 ng/dL showed poor response to novel AR antagonists, but better response and efficacy to the treatments of Docetaxel and Cabazitaxel. Bipolar androgen therapy (BAT) can make CRPC sensitive to subsequent ADT. The sequential treatment of BAT and enzalutamide showed the potential to significantly improve the survival and prognosis of men with CRPC.

Based on the evidence, the dynamic of serum TST level provide a significant role in advanced prostate cancer patients who received ADT.

Keywords: testosterone, prostate cancer, prognosis, treatment effect, bipolar androgen therapy

Introduction

One of the most prevalent cancers affecting the male genitourinary system is prostate cancer (PC) [1]. With 31,620 predicted fatalities in 2019, it continues to be the second most typical reason for cancer deaths among men in the United States [2]. An important turning point in the history of prostate cancer treatment occurred in 1941, when Dr. Charles Huggins discovered that androgen deprivation therapy (ADT) offered considerable palliative benefits for men with advanced prostate cancer [3].

Throughout the rest of history, ADT has remained the preferred prostate cancer therapy. Nevertheless, a number of studies have demonstrated that men who get ADT will inevitably develop castration-resistant prostate cancer (CRPC) [4, 5]. This is attributed, according to several theories [6-10], to persistent androgen receptor (AR) signaling. Newer oral medications that target the androgen axis of prostate cancer, such as the androgen receptor antagonist enzalutamide and the cytochrome P450 17A (CYP17A) inhibitor abiraterone, have been introduced into the clinic, improving overall survival in men with CRPC [11-15].

Gradual Progress of Androgen Therapy for Prostate Cancer

At present, it appears that practically all methods of treating prostate cancer involve lowering serum testosterone and reducing androgen receptor signaling

(ARS). But, not to be overlooked, Huggins also proposed that treating prostate cancer with excessive androgens, a method he coined "hormone interference," would be effective in treating prostate cancer [16]. This shows that there may be a positive association between androgens and prostate cancer. Supra-physiologic levels of androgens have been shown to impede the proliferation of AR-positive human CRPC cell lines [17, 18]. In certain investigations, a number of potential explanations underlying this paradoxical impact have been clarified. Isaac demonstrated that AR is a licensing factor for DNA replication, plays a key role in DNA replication, and must be degraded as cells go through the cell cycle [18-21]. The enhanced ligand-bound AR in the nucleus is permanently present without degrading in the presence of supraphysiologic testosterone. DNA replication and relicensing are prevented by insufficient AR degradation, which causes cell death in succeeding cycles [20]. Bipolar androgen therapy (BAT), a treatment for CRPC, was developed as a result of the identification of these mechanisms [22].

Additionally, a significant number of findings were unexpected given the androgen hypothesis. For instance, there is no connection between prostate volume, PSA, endogenous testosterone levels, or prostate cancer [23-26], and analyses of population studies have discovered that not all naturally occurring testosterone levels are related to prostate cancer [24]. In addition, several lines of evidence have shown that reduced serum total testosterone levels and reduced free testosterone levels are associated with more aggressive PC and worse prognosis [27-30]. The findings also appear to suggest that the relationship between testosterone and PC is not a simple linear relationship and that there is a certain threshold associated with the onset and progression of cancer at various stages, in addition to the previously mentioned conflicting antitumor effects of various serum androgen levels.

Therefore, we searched the recent relevant literature and combined it with our clinical findings. The relationship between serum testosterone levels and different stages of prostate cancer was reviewed.

Serum Testosterone and Prostate Cancer

The association between testosterone and prostate cancer in the past was primarily based on the idea that testosterone provides "fuel" and "energy" for prostate cancer cells. Following ADT therapy, the testosterone levels of PC patients dropped, leading to a significant number of patients with testosterone deficiency (TD). TD can cause a series of worrying health problems [31], and

testosterone replacement therapy (TRT) is the preferred treatment at present. TRT has been shown to improve or even reverse these symptoms [32, 33].

In the eyes of researchers, it opens the door to the use of testosterone replacement therapy (TRT) for patients with prostate cancer, but it also raises serious ethical and medical concerns.

1. *Androgen saturation model explains the paradoxical relationship between testosterone and prostate cancer.*
   According to some studies, the highest (saturation) level of testosterone binding to AR takes place at relatively low concentrations [34, 35]. Low testosterone levels can affect PC negatively. The range of testosterone levels that can affect the PC in this setting is extremely constrained because once the ARs are fully occupied, excess testosterone cannot enter the cell to stimulate cell growth. Prostate tissue is sensitive to changes in testosterone levels at low concentrations but not at high concentrations [36, 37]. Young, healthy men with elevated serum total and free testosterone did not show elevated serum or semen PSA levels or increased prostate volume [38, 39]. Similar results were also seen in elder men [40].
2. *Testosterone replacement therapy (TRT) is safe and beneficial for TD patients with prostate cancer.*
   Firstly, TRT does not increase the risk of prostate cancer in healthy individuals [41, 42]. Second, TRT does not encourage the progression or recurrence of early-stage prostate cancer. Following radical prostatectomy (RP), PC patients treated with various TRTS have not demonstrated any biochemical or clinical recurrence [43, 44]. Similarly, TRT caused no signs of PC recurrence or progression in prostate cancer patients receiving radiotherapy [45, 46].
   There is proof that men with prostate cancer who are receiving TRT have a lower overall biochemical recurrence rate than those in the control group [47-49]. The increased androgen levels with TRT may have a protective effect on the recurrence of PC. These connections could point to a biological mechanism by which testosterone influences the differentiation and operation of healthy prostate epithelial cells. High or normal levels of testosterone may keep prostate and early PC cells in a well-differentiated state. Conversely, prostate cancer cells may become less differentiated and more malignant as a result of a gradual drop in testosterone brought on by advanced age or disease [50].

Men with PC and TD have a higher risk of disease aggressiveness [51]. Finally, low serum testosterone levels did not independently predict prostate cancer bone metastasis [52].

These offer a fresh approach to treating PC patients and point us in the right direction for research on the connection between serum testosterone and prostate cancer. High physiological testosterone levels are preventative for prostate cancer. Prostate cancer does not progress or recur after testosterone supplementation in PC patients [43-45, 48, 53-55].

Serum Testosterone and Androgen Deprivation Therapy (ADT)

Previously, lower TST levels in patients who received ADT have been associated with a longer response of durations [56, 57]. The target TST level during ADT for prostate cancer is defined as less than 50ng/dl by current recommendations [58]. The target of 50 ng/dl has been contested, though, as more precise assays have been developed.

The clinical importance of a reduced TST in ADT has been reported in a number of studies. For the first time, Morote described the clinical significance of lower castration levels. They noted that the clinical significance of breakthrough TST increased at 20 and 50 ng/ dL and suggested that no breakthrough is a good predictor of survival in androgen-independent progression [57]. According to Perachino, TST at 6 months (40 ng/dl) was directly related to the risk of death during ADT [59]. After discussing OS and TST levels following six months of ADT, Bertaglia concluded that a TST level of less than 30 ng/dl was a positive prognostic factor for survival [56]. However, because patients with TST levels under 20 ng/dl rarely experienced fatal outcomes during the study, they were unable to fully evaluate lower TST levels. The median nadir TST was also 39 ng/dL, which is significantly higher than the data from our team (median minimum TST for the past six months was 13 ng/dl). This might be caused by variations in ADT protocols and patient traits like ethnicity, the prevalence of advanced cancer, and first-line local regional treatment.

Data from Japanese patients who received ADT as their initial prostate cancer treatment were retrospectively examined by our team [60, 61]. Significant prognostic factors included a nadir serum testosterone level of less than 20 ng/dL and a testosterone reduction of more than 480 ng/dL [60]. Additionally, based on the intervals before and after 6 months in which nadir

testosterone was less than 20 ng/dl, patients were divided into two groups: fast and slow. Between the two groups, there was no discernible difference in overall survival. The prognosis of ADT patients may depend less on the rate of testosterone decline and more on whether the lowest testosterone level is below 20 ng/dL [61].

Testosterone and Bipolar Androgen Therapy

The term "bipolar" is used to emphasize that, with this strategy, there is a rapid cycle of testosterone between two extremes: from supraphysiologic serum testosterone levels back to levels near castration, repeated over multiple cycles. Due to their inability to completely degrade high levels of androgen-stabilized nuclear AR, CRPC cells that express high levels of AR are vulnerable to cell death when exposed to supraphysiologic testosterone. Supraphysiologic androgens can also cause deadly double-stranded DNA breaks in prostate cancer cells that have been chronically deficient in androgens. CRPC cells that have survived high testosterone levels due to low baseline AR levels or through adaptive downregulation of AR become susceptible to death when suddenly re-exposed to low testosterone during the treatment cycle because of the bipolar nature of the treatment [62].

A study [63] has shown that androgens can express the 'hit and run' mechanism in prostate cancer cells through androgen receptors. In a cell-autonomous manner, androgens can cause prostate cancer cells to maintain a quiescent and dormant state. Therefore, by inducing and/or strengthening self-sustaining quiescent cancer cells in disseminated solitary tumor foci, androgen deprivation and supplementation of the repeated cycle [i.e., bipolar androgen therapy (BAT)] can effectively inhibit tumor cells in the early stage of metastatic progression.

One of the factors that allows for DNA replication in prostate cancer is the androgen receptor (AR). During androgen ablation therapy and the development of prostate cancer into mCRPC, the expression of the AR protein was dramatically increased (50–100 folds). Nuclear AR in mCRPC cells binds to DNA at the origin of replication sites (ORS) during the G1 phase of the cell cycle as a component of the replication origin complex (ORC), which is necessary to allow DNA replication during the S phase.

From early mitosis until late mitosis, AR and ORC are linked. It must be degraded as a DNA licensing factor in order for re-licensing to take place in the following cell cycle. The increased ligand makes the AR bound by ORC excessively stable and prevents its complete degradation when there are medications present to supplement serum testosterone. Lack of sufficient mitotic AR degradation prevents DNA replication from restarting due to the ligand-dependent over stability, which causes cell death in the subsequent circulation [18-20] (Figure 1).

In the first BAT pilot study conducted by Schweizer in 2015, 16 asymptomatic mCRPC patients completed BAT for at least 3 months. The study showed that 50% of patients had a decrease in PSA, of which 28.6% had a decrease of more than 50%. Some soft tissue metastases were controlled in 10 patients according to imaging evaluation [64].

BAT was linked to appreciable gains in lipid parameters, quality of life, and body composition. For men with mCRPC, this has positive implications for their long-term health [65]. Systemic pain and calf swelling were the most frequent BAT side effects in the RESTORE study, which involved 90 patients. Hot flashes, breast tissue enlargement, and breast pain are typical sexual side effects [66] (Table 1).

It is worth mentioning in particular that a large (n = 180) randomized trial of BAT (TRANSFORMER) [71] compared the clinical or imaging progression free survival (PFS), safety, and quality of life (QoL) of asymptomatic anti-castration metastatic prostate cancer patients treated with bipolar androgen and enzalutamide.

Compared with enzalutamide, BAT maintains or improves the quality of life, especially in the areas of fatigue, physical and sexual function. The experiment also made a comparison of cross-treatment. Patients who were cross treated with enzalutamide after BAT showed a significantly enhanced response compared to patients who received enzalutamide immediately after the progression of abiraterone. The PSA-PFS of enzalutamide increased nearly threefold from 3.8 months after Abiraterone to 10.9 months after BAT. PSA50 response improved to 78% versus 25%, and OR improved to 29% versus 4%. This suggests that BAT may partially reverse the lineage plasticity of PC cells that lose AR addiction. In other words, BAT can reverse anti-androgen resistance through adaptive down-regulation of AR expression [71].

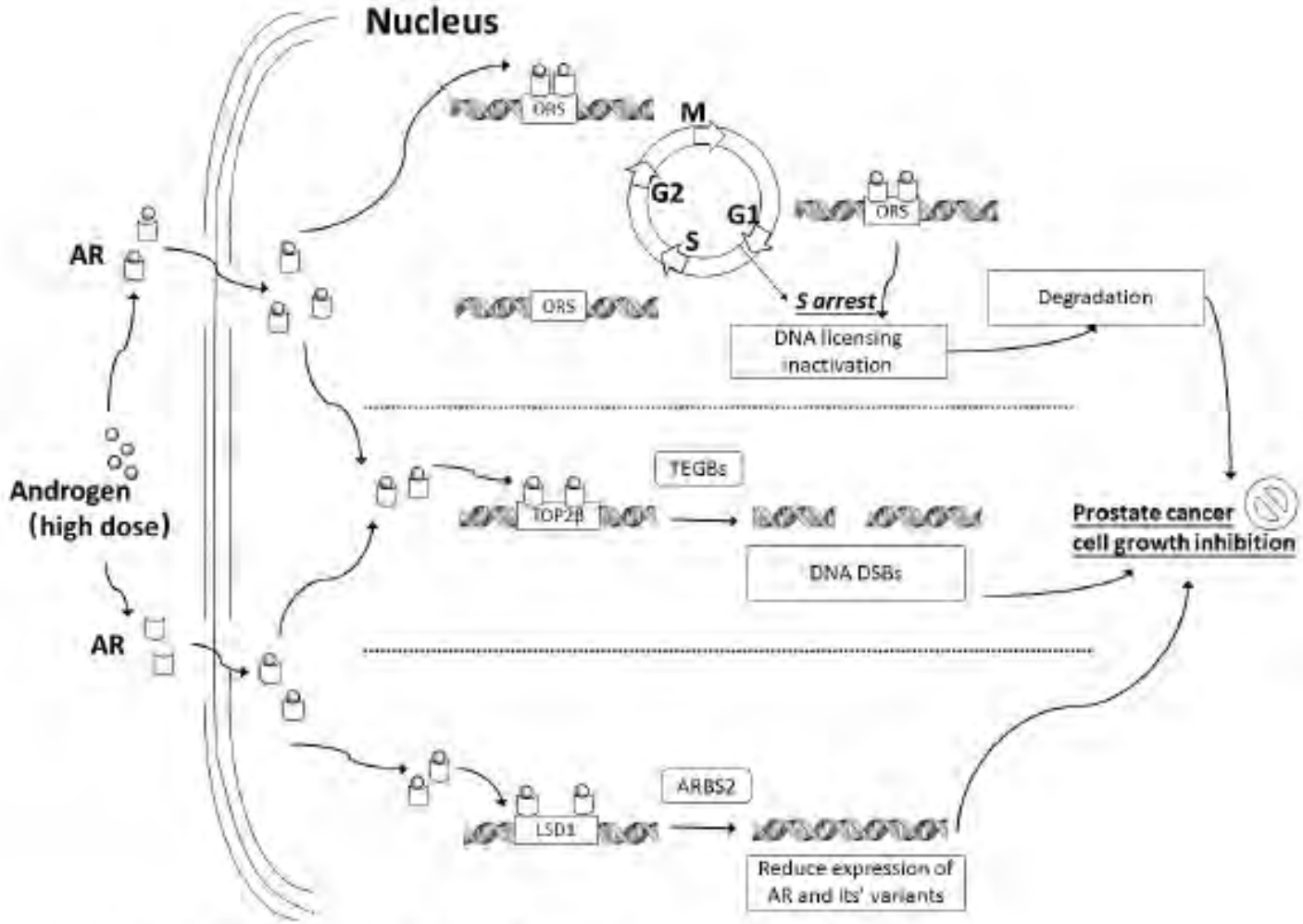

Figure 1. Mechanism of high dose androgen inhibiting the growth of prostate cancer cells.

Table 1. Summary of current BAT research

Patient status	Number	Treatment plan	Result	Reference
CRPC	12	Testosterone 5 mg transdermal patch or 1% gel for 1 week or 1 month.	30% of patients showed decreased PSA.	[67]
Early CRPC with micrometastasis	15	25, 50, or 75 mg/day transdermal testosterone.	Symptom progression in one patient, PSA decrease in three patients	[68]
Asymptomatic CRPC with low to moderate metastasis	16	Testosterone (400 mg intramuscular injection on the first day of the 28-day cycle) and etoposide (100 mg orally per day; days 1 to 14 of the 28-day cycle)	PSA decreased in half of the patients, and imaging regression occurred in half of the 10 patients with assessable soft tissue metastasis.	[64]
HSPC with low metastasis	29	Testosterone 400 mg intramuscularly on days 1,29, and 57	PSA level < 4 ng/mL in 17 patients at 18 months	[69]
mCRPC developed after enzalutamide	30	Alternatively use BAT cycle for 3 months (400 mg intramuscular injection on the 1st, 29th or 57th day), and then use ADT alone for 3 months	30% of patients achieved PSA decrease; 52% of patients recovered sensitivity to enzalutamide treatment (PSA decreased)	[70]
mCRPC (duration of abiraterone < or ≥ 6 months)	180	Testosterone 400 mg, intramuscularly once every 28 days or enzalutamide 160 mg per day, until clinical or imaging progress. Asymptomatic patients enter the cross-treatment link after the 28-day clearance period.	The PSA-PFS of enzalutamide increased nearly threefold, from 3.8 months after abiraterone to 10.9 months after BAT.	[71]

Another phase II BATMAN study evaluated the efficacy of alternating BAT and ADT in men with recurrent or advanced hormone-sensitive prostate cancer. Twenty-two (76%) patients in the study remained sensitive to castration after two rounds of BAT-ADT. Five of the seven nonresponders who progressed to CRPC at the end of the study responded to subsequent antiandrogenic therapy (using bicalutamide or enzalutamide) [72]. Other studies have also shown that BAT treatment can induce clinical responses and restore the sensitivity of previously treated CRPC patients to androgen receptor ablation [66, 71, 73, 74].

In addition, it is reported that the combination of BAT and enzalutamide may improve the clinical response rate of mCRPC patients to blocking PD-1 at the immune checkpoint [75].

Serum Testosterone Determines CRPC Drug Therapy

It should be noted that there is also a close relationship between serum TST levels and responses to novel AR-targeted drugs [76]. The level of serum TST is expected to determine the best treatment strategy for patients with CRPC.

Table 2. Serum testosterone determines CRPC drug therapy

Serum Testosterone	Influence of Drug Therapy
TST ≥ 13 ng/dL	Better outcomes in Enzalutamide and/or Abiraterone. Good response to new AR-targeted drugs
TST< 13ng/dL	Poor response to novel AR antagonists,
	Better response and efficacy to Docetaxel

Our team studied the relationship between serum testosterone and treatment response and prognosis in patients treated with enzalutamide and Abiraterone. Studies have shown that higher TST levels (≥13 ng/dL) are associated with better outcomes in Enzalutamide and/or Abiraterone treated patients. The TST level of 13 ng/dL can predict the good response of CRPC patients to new AR-targeted drugs. Higher TST (≥13 ng/dL) at the beginning of an administration is related to a good response to new AR-targeted drugs, especially Enzalutamide [76]. Serum TST 13 ng/dL is the dividing point that determines the response and efficacy of CRPC to drug therapy [77]. Patients with serum TST≥13ng/dL had better curative effects on novel androgen receptor (AR) antagonist medicines. However, those serum TST< 13ng/dL

showed poor response to novel AR antagonists, but better response and efficacy to the treatments of Docetaxel and Cabazitaxel [76, 77] (Table 2).

Conclusion

The relationship between prostate cancer and androgen has changed from the original single understanding in recent years due to the advancement and development of pertinent research. Of course, there are still a lot of unanswered questions regarding androgen and prostate cancer despite the abundance of basic analyses and clinical reports. For instance, the dosage of testosterone treatment and the proper BAT cycle.

Traditional hormone therapy and anti-cancer medications have been replaced by new hormone drugs that target the BRCA1/2 mutation and PARP inhibitors in the treatment of prostate cancer. Serum testosterone, however, continues to be a crucial biochemical factor that affects the effectiveness of new prostate cancer drug therapies as well as the prognosis of patients with the disease. It also plays a small but significant role in the proliferation and apoptosis of prostate cancer cells.

Simply put, the serum testosterone level is a helpful biochemical indicator for determining the treatment course and prostate cancer prognosis. Serum testosterone has a significant impact on the treatment, prognosis, and quality of life of patients with advanced prostate cancer in clinical practice.

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International Journal of
Molecular Sciences

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Review

Contribution of the L-Type Amino Acid Transporter Family in the Diagnosis and Treatment of Prostate Cancer

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Abstract: The L-type amino acid transporter (LAT) family contains four members, LAT1~4, which are important amino acid transporters. They mainly transport specific amino acids through cell membranes, provide nutrients to cells, and are involved in a variety of metabolic pathways. They regulate the mTOR signaling pathway which has been found to be strongly linked to cancer in recent years. However, in the field of prostate cancer (PCa), the LAT family is still in the nascent stage of research, and the importance of LATs in the diagnosis and treatment of prostate cancer is still unknown. Therefore, this article aims to report the role of LATs in prostate cancer and their clinical significance and application. LATs promote the progression of prostate cancer by increasing amino acid uptake, activating the mammalian target of rapamycin (mTOR) pathway and downstream signals, mediating castration-resistance, promoting tumor angiogenesis, and enhancing chemotherapy resistance. The importance of LATs as diagnostic and therapeutic targets for prostate cancer was emphasized and the latest research results were introduced. In addition, we introduced selective LAT1 inhibitors, including JPH203 and OKY034, which showed excellent inhibitory effects on the proliferation of various tumor cells. This is the future direction of amino acid transporter targeting therapy drugs.

Keywords: prostate cancer; LAT1; LAT3; diagnosis; treatment

Citation: Zhao, X.; Sakamoto, S.; Wei, J.; Pae, S.; Saito, S.; Sazuka, T.; Imamura, Y.; Anzai, N.; Ichikawa, T. Contribution of the L-Type Amino Acid Transporter Family in the Diagnosis and Treatment of Prostate Cancer. *Int. J. Mol. Sci.* **2023**, *24*, 6178. https://doi.org/10.3390/ijms24076178

Academic Editors: Domingo González-Lamuño and Raquel Yahyaoui

Received: 27 February 2023
Revised: 20 March 2023
Accepted: 22 March 2023
Published: 24 March 2023

1. Introduction

Tumor growth requires continuous nutritional support, of which essential amino acids (EAAs) are an important source [1]. The amino acid uptake was found to be higher in tumor tissue than in normal tissue [2]. The L-type amino acid transporter (LAT) family is a group of transmembrane transporter proteins composed of four members, LAT1, 2, 3, 4. The first two proteins belong to the solute vector family 7 (SLC7) and the latter two belong to SLC43. They are important pathways for essential amino acids to enter cells and are closely related to intracellular pathways [3–6].

The LAT family was upregulated in many tumors [7]. Prostate cancer (PCa) is the most frequent malignant tumor in men globally. In the United States, approximately 2.6 million new cases of PCa were identified each year, with an estimated 34,500 fatalities [8]. We primarily reviewed the relationship between the LAT family and PCa and analyzed the role of LATs in the diagnosis and treatment of PCa.

2. The Structure and Function of LATs

2.1. LAT1

LAT1 (SLC7A5) was discovered in 1998 [3,9]. It combines with 4F2hc (SLC3A2) heavy chain protein to form a transmembrane complex to play its biological role [10]. LAT1 is mainly responsible for the transport of large neutral amino acids. Generally, LAT1 is expressed in the normal human body mainly in the gastrointestinal mucosa,

testicular support cells, ovarian follicular cells, pancreatic islet cells, and endothelial cells that act as inter-tissue barriers (blood-brain, blood-retina, and blood-follicular barriers) [11]. Many neurotransmitters and neuroactive compounds cannot cross the blood-brain barrier, and most of them are synthesized in the brain. Therefore, LAT1, as the precursor of neurotransmitters and neuroactive compounds, plays an important role in amino acid uptake through the blood-brain barrier [12]. As previously stated, LAT1 expression in normal organisms is limited to specific cells and tissues. Studies have also shown that LAT1 exists in cells with high proliferation and differentiation ability, such as embryos and T-lymphocytes. During embryonic development, the LAT1 of syncytial trophoblast cells plays an indispensable role in the development of the placenta, contributing to the exchange of amino acids between mother and fetus. Systemic LAT1 knockout results in defects in the placenta, which can be fatal to the embryo in the second trimester [13]. There is no clear research result on the role of LAT1 in T cell differentiation, but previous studies have shown that LAT1 is related to the metabolic process of T lymphocyte differentiation. LAT1 expression is low in intrinsic T cells, whereas T cells at the differentiation stage specifically increase LAT1 expression by T cell receptors (TCR), ensuring sufficient nutrients to react with the antigen [10,14].

LAT1 provides branched-chain amino acid (BCAA), especially leucine, to the mammalian target of rapamycin complex1 (mTORC1), a major control factor of cell proliferation. mTORC1 senses amino acid signals and promotes cell proliferation through multiple downstream effectors related to gene expression and metabolism [15]. Upstream of mTORC1 is a complex of GTPase-activating protein (GAP), activity toward Rags 1 (GATOR1) and GATOR2. GATOR1 functions as a mTORC1 antagonist and GATOR2 functions as a mTORC1 agonist. GAP inhibits mTORC1 in the absence of amino acids. Leucine transports into the cell by LAT1 binds to the leucine sensor sestrin2. The interaction between leucine and sestrin2 can activate GATOR2 and inhibit GATOR1, thus promoting the function of mTORC1 to achieve the purpose of cell proliferation [16,17]. LAT1 expression is upregulated in many cancers, such as breast [18], lung [19], colorectal [20], renal [21], bladder [22], prostate [23], and gliomas [24].

One of the purposes of large quantities of amino acid transport in LAT1 in cancer cells is to use BCAAs as biosynthetic materials for the metabolic reprogramming of cancer cells. Branched-chain amino acid transferase (BCAT) deaminates free BCAAs to generate the appropriate branched-chain keto acid (BCKA). BCAT2 transforms BCAA into BCKA within the mitochondria, which is subsequently catalyzed by metabolic intermediates and acetyl-CoA into the TCA cycle, which is used for energy production and fatty acid metabolism [25] (Figure 1).

Another function of LAT1 that promotes tumor formation is to inhibit cell-damaging T-cell control in the tumor microenvironment. In the serine pathway, tryptophan is converted into kynurenine (Kyn) by 2, 3-Deoxygenase (TDO) and indoleamine 2, 3-Dioxygenase (IDO). In the physiological state, TDO and IDO levels are negligible, but in the cancer state, both enzymes rise dramatically. Through the continuous function of TDO and IDO, large amounts of Kyn can be synthesized. Kyn is transported from cancer cells to T cells by LAT1. In T cells, Kyn binds to the aryl hydrocarbon receptor (AHR), which inhibits the anti-tumor immune response of T cells and promotes the proliferation of cancer cells [26].

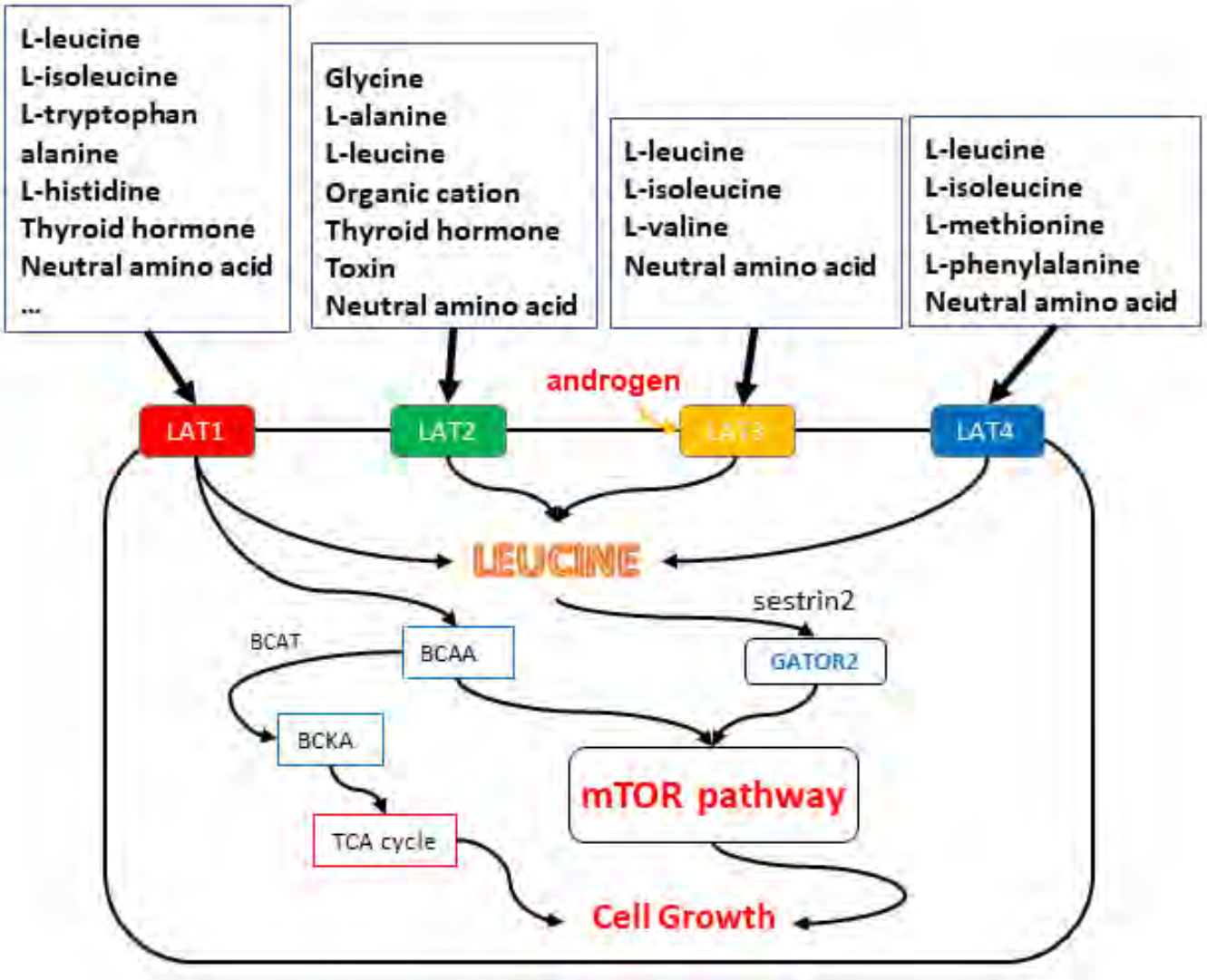

Figure 1. The substances that each LAT is mainly responsible for transporting are shown in the figure.

Figure 1. The substances that each LAT is mainly responsible for transporting are shown in the figure. LATs provide branched-chain amino acids (BCAA), especially leucine, to mammalian target cells of rapamycin complex 1 (mTORC1), and the mTOR pathway is a major control factor in cell proliferation. Leucine is transported into the cell by LATs, binds to the leucine sensor sestrin2, and GATOR2 acts as mTORC1 agonist. Thus, it can promote the function of the mTOR pathway and achieve the purpose of cell proliferation. In addition, LAT1 can use BCAAs as biosynthetic materials for the metabolic reprogramming of cancer cells. BCAT deaminates free BCAAs to form BCKA. It enters the TCA cycle and is used for energy production and fatty acid metabolism to promote cell proliferation.

2.2. LAT2

LAT2 (SLC7A8) was discovered in 1999 [4,27,28]. The structure of LAT2 is similar to that of LAT1, and it forms a transmembrane complex with 4F2hc to mediate transport [29]. However, LAT2 has a wider range of substrate specificity than LAT1, including polar uncharged amino acids and small neutral amino acids [4]. LAT2 commonly expresses in normal humans [11] and is associated with the development of amino-aciduria [30]. Both LAT1 and LAT2 consist of 12 transmembrane domains that form pathways for their substrates [29]. They bind to the heavy glycoprotein subunit 4F2hc via disulfide bonds. Although 4F2hc does not have direct substrate transport function [31], it enables more stable localization of LAT1 and LAT2 on the plasma membrane [32]. Previous studies have suggested that 4F2hc lacks amino acid transport activity. Instead, the combined LAT1 and LAT2 units are the only ones with transport capacity [31]. But now, there are different

Another function of LAT1 that promotes tumor formation is to inhibit cell damag
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LAT2 (SLC7A8) was discovered in 1999 [4,27,28]. The structure of LAT2 is simila

views on it. 4F2hc acts as a molecular chaperone to enable LAT1 and LAT2 to become its final site on the membrane [32]. 4F2hc is required for the transport of LAT1 and LAT2 to the plasma membrane [9], where LAT1 and LAT2 are thought to determine the transport properties of heterodimers. At the same time, increased 4F2hc expression levels in many forms of cancer are associated with poorer prognosis in several studies [33–36] (Figure 2).

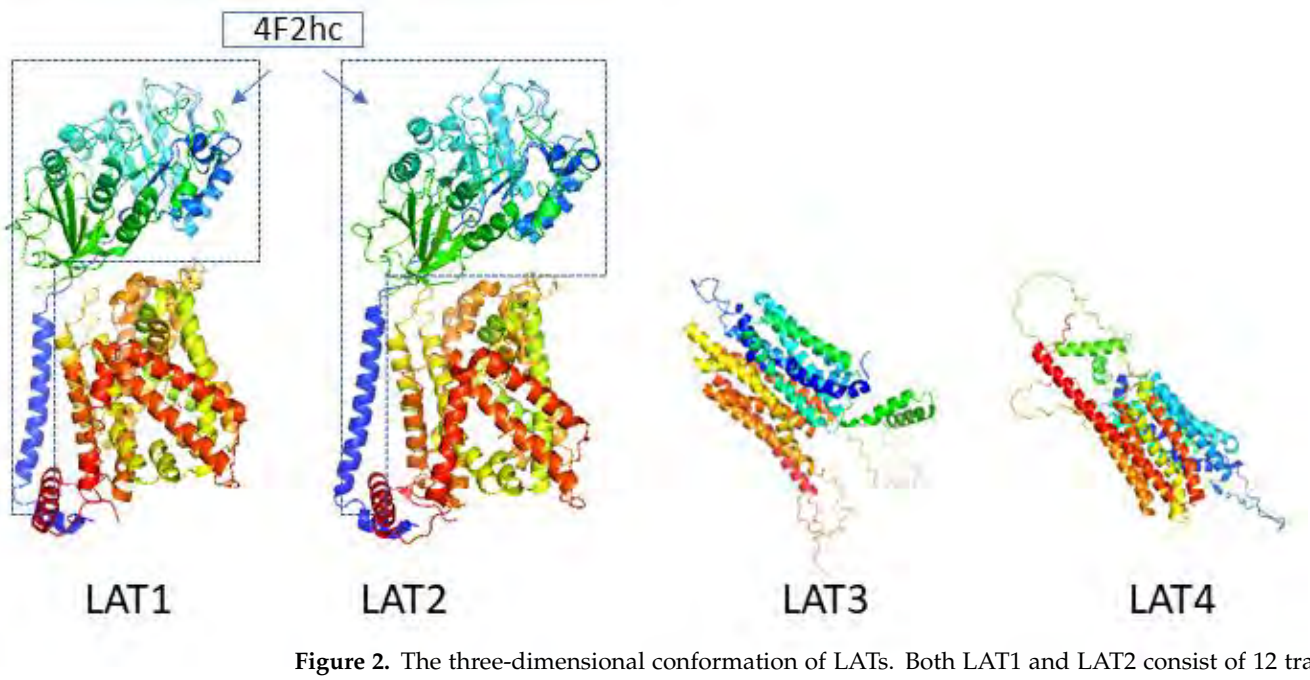

Figure 2. The three-dimensional conformation of LATs. Both LAT1 and LAT2 consist of 12 transmembrane domains that form pathways for their substrates. They bind to the heavy glycoprotein subunit 4F2hc via disulfide bonds. Unlike LAT1 and LAT2, the biological functions of LAT3 and LAT4 do not require binding to heavy chains and can exist independently. (Images created using PyMol*, colord by chainbows, the PDB ID: 6IRS, 7CMI, the Uniprot ID: O75387, Q8N370, PyMol* (version 2.5 Schrodinger. Warren L. DeLano), and RCSB PDB, and UniProt).

2.3. LAT3

LAT3 (SLC43A1) was first named POV1, "Prostate cancer Overexpressed gene 1", and it was upregulated in prostate cancer as a gene of unknown function [37]. It was really discovered definitively in 2003 [5]. LAT3 is usually expressed in the liver, skeletal muscle, and pancreas [38]. Overall LAT3 expression is low in the kidney, but LAT3 expression is stronger in the apical plasma membrane of the podocyte foot processes. It shows that LAT3 is important for the development and maintenance of podocyte function and structure [39]. Another study has also shown that LAT3 expression is required for erythrocyte development to produce hemoglobin [40]. LAT3 can be upregulated in response to androgen, which is closely related to leucine uptake and cell proliferation in human prostate cancer cell lines [41].

2.4. LAT4

LAT4 (SLC43A2) was discovered in 2005 and identified by homology with LAT3 [6]. LAT4 is usually expressed in epithelial cells of the small intestine, proximal renal tubules, and thick ascending limbs [6]. However, in mouse models, LAT4 is expressed in the intestine, kidney, brain, white adipose tissue, testis, and heart, but not detected in the liver, showing differences from human expression [6]. The physiological function of LAT4 in these organs is still not fully understood. In LAT4 knockout mice, newborn mice are smaller than wild-type mice, suggesting that LAT4 is important for growth and development [42]. Unlike LAT1 and LAT2, the biological functions of LAT3 and LAT4 do not require binding to heavy chains and can exist independently. LAT3 and LAT4 are both sodium-dependent neutral amino acid transporters. The exact mechanism of transport for LAT3 and

LAT4 is not well understood, but it is thought to involve a symport mechanism, in which the transport of amino acids is coupled to the uphill movement of sodium ions against their concentration gradient. The movement of ions and amino acids is driven by the energy generated from the electrochemical gradient established by the sodium/potassium ATPase. The difference between LAT3 and LAT4 lies in their substrate specificity and tissue distribution. LAT3 has a higher affinity for large neutral amino acids, such as leucine and isoleucine [5], while LAT4 has a higher affinity for small neutral amino acids, such as alanine and serine [6] (Figures 1 and 2).

3. LATs and PCa

LATs promote PCa progression in several ways:

1. Amino acid uptake: LATs increase the uptake of essential amino acids into cancer cells, which supports their growth and survival. After being delivered into cells, these amino acids are used to make proteins, nucleic acids, lipids, and ATP. Compared with normal cells, cancer cells have higher upregulation transporters (LATs), which can promote the entry of foreign amino acids into cells, and the stable acquisition of amino acids by cancer cells is important for cancer growth. By increasing the availability of amino acids, LATs can promote PCa cell proliferation and invasion.
2. Activation of signaling pathways: LATs have been shown to activate various signaling pathways, including the mTOR pathway, which is involved in the regulation of cell growth, proliferation, metabolism, and survival. LAT1 [23] and LAT3 [41] are highly expressed in prostate cancer, providing branched-chain amino acids (BCAA) to the mammalian target protein of rapamycin complex (mTORC1), which senses amino acid signaling and promotes cell proliferation through multiple downstream effectors related to gene expression and metabolism [15]. Leucine, which enters the cell via LAT1, binds to the leucine sensor sestrin2. The interaction between leucine and sestrin2 can activate GATOR2 and inhibit GATOR1, thus promoting the function of mTORC1 and achieving the purpose of cell proliferation [16,17]. LATs can regulate mTOR activity by influencing the availability of essential amino acids, such as leucine, that activate the pathway.
3. Drug resistance: In PCa cells resistant to antiandrogen therapy (ADT), the expression of some LATs is up-regulated, which may promote the progression of PCa to castration-resistant prostate cancer (CRPC) through androgen receptor variants [43]. Changes in the microenvironment induced by hormone deprivation therapy can alter the expression of LAT1 and LAT3. Reduced androgen receptor signaling may lead to decreased LAT3 expression and, as another consequence, increased LAT1 expression. Changes in the microenvironment induced by hormone deprivation therapy can alter the expression of LAT1 and LAT3. Decreased androgen receptor signaling may lead to decreased LAT3 expression and, as another consequence, the production of the AR-V7 variant, resulting in increased 4F2hc expression, and decreased leucine, resulting in increased LAT1 expression. The two form a dimer that eventually causes leucine to be re-transported into the cell to promote cancer cell proliferation. PCa is transformed into CRPC, which is resistant to ADT treatment.
4. Promotion of angiogenesis: LATs have been implicated in the regulation of blood vessel formation (angiogenesis), which is essential for the growth and spread of PCa cells. Tumors grow and evolve through constant crosstalk with the surrounding microenvironment. New evidence suggests that angiogenesis and immunosuppression often occur together in response to this crosstalk [44]. For example, the expression of LAT1 was significantly correlated with the expression of VEGF, CD34, and microvascular density at the primary and metastatic sites [45–48]. VEGF and CD34 are factors related to angiogenesis. LAT1 can also mediate the angiogenesis of miR-126 on primary human pulmonary microvascular endothelial cells by regulating mTOR signaling [49].

5. Others: Increase the uptake of amino acids by inducing hemoglobin maturation [40]. LAT3 expression is required for the development of red blood cells to produce hemoglobin. LAT3 can be upregulated under the action of androgens [41], which leads to the increase of hemoglobin development and increases the way for tumor cells to obtain nutrients from another side, thus promoting their proliferation.

Current studies on prostate cancer and LATs usually focus on LAT1 and LAT3. There are few studies on LAT2 and LAT4. Studies show the main leucine transporters are different in different stages of prostate cancer, especially in the castration-resistant stage of androgen receptor (AR) expression [23,50]. Studies have found that LNCaP cells mainly express LAT3, while LAT1 is mainly expressed in DU145 and PC-3 cells [23,50]. According to Rii's study, LAT3 is highly expressed in LNCaP and C4-2 cells expressing AR, but hardly expressed in AR-negative PC3 and DU145 cells [51]. Changes in the microenvironment, such as starvation or hormone deprivation, can promote cancer formation and alter LAT1 and LAT3 expression. Reduced androgen receptor signaling may lead to decreased LAT3 expression and, as another consequence, increased LAT1 expression [41].

In addition to the common relationships mentioned above, we will describe the relationship between LAT1, 2, 3, 4, and prostate cancer respectively.

3.1. LAT1

It has been found that the up-regulation of LAT1 during antiandrogen therapy (ADT) promotes the progression of PCa cells. LAT1 is highly expressed in CRPC cell lines. LAT1 knockdown significantly reduces cell proliferation, migration, and invasion. High LAT1 expression is associated with poor biochemical recurrence-free periods in patients treated with chronic ADT [23]. Another study also confirmed that LAT1 expression is up-regulated at the protein and mRNA levels in 22Rv1 CRPC tumors with chronic ADT [52]. Sugiura demonstrated a potential relationship between AR-V7 and the LAT1-4F2hc complex. AR-V7 activates downstream target genes in the absence of androgens. 4F2hc is one of the downstream target genes of AR-V7. The expression level of 4F2hc in CRPC tissues is significantly increased, which correspondingly suggests poor prognosis of related patients [43]. Furthermore, a study shows ATF4 gene expression is upregulated during metastasis, suggesting that ATF4-mediated amino acid response element-containing gene regulation may be important for the development of metastatic CRPC [53]. ATF4-regulated genes, such as LAT1 and 4F2hc, show low expression in normal prostate tissue and primary prostate cancer, but they are significantly increased in metastatic prostate cancer, suggesting that these transporters are involved in the nutrient supply required for metastatic prostate cancer [53] (Figure 3).

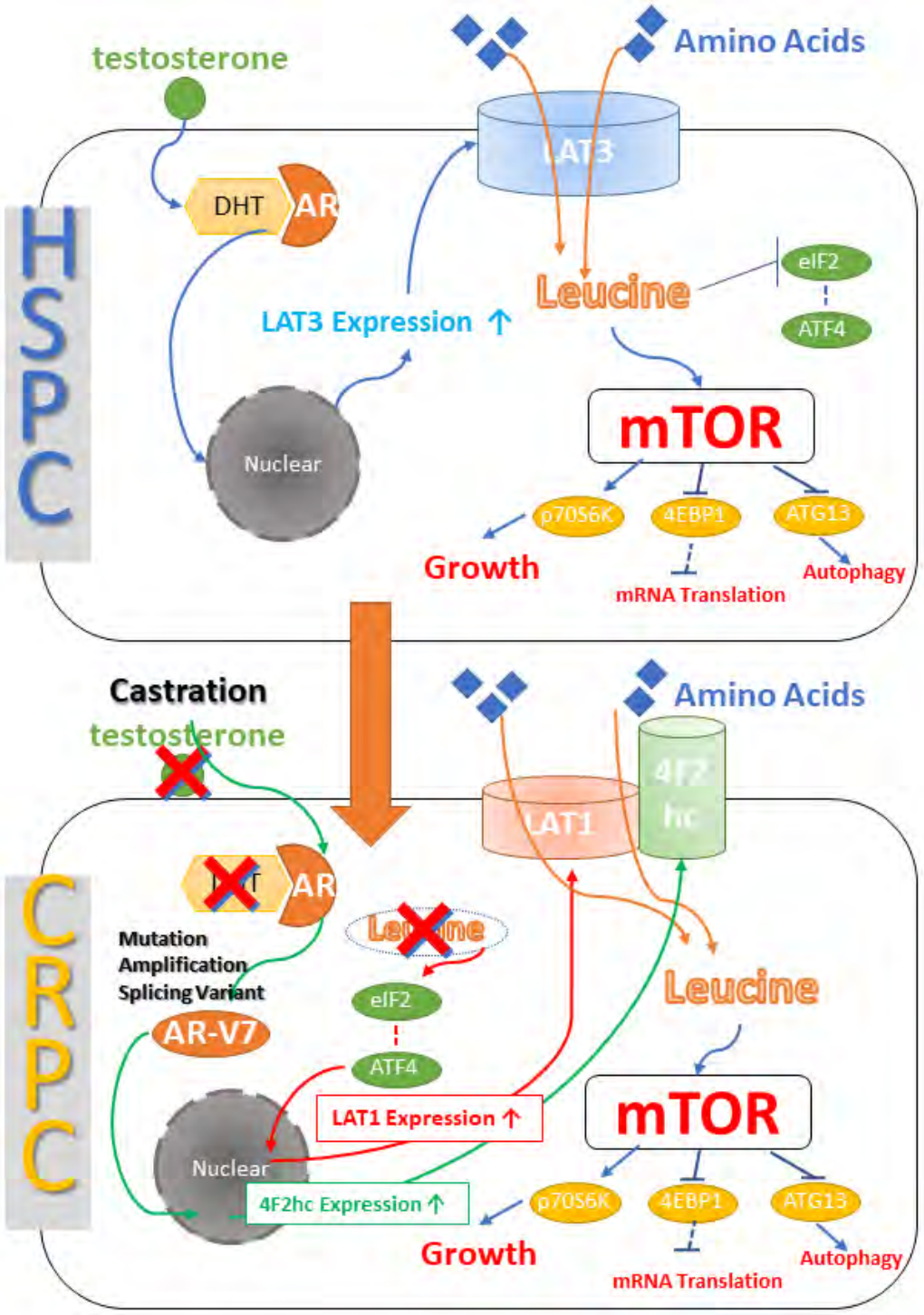

Figure 3. LAT is linked to both hormone-sensitive prostate cancer (HSPC) and castration-resistant prostate cancer (CRPC). In untreated HSPC, 5-alpha reductase converts testosterone to dihydrotestosterone (DHT), which binds to the androgen receptor (AR), enters the nucleus, and stimulates LAT3 transcription, resulting in enhanced LAT3 expression and contributes to the mTORC1 pathway activation. When hormone therapy is used to treat PCa, testosterone levels fall, resulting in castration, and ARs that no longer bind DHT change, increase, and generate splicing variants. AR-V7, in particular, can enter the nucleus in the absence of testosterone activation, and 4F2hc is present in its downstream signaling. Furthermore, the removal of leucine from the cells results in the lack of eIF2 repression and the admission of ATF4 into the nucleus. It enhances LAT1 expression, and LAT1 and 4F2hc form a dimer, allowing leucine into the cell and promoting tumor cell proliferation [54].

Figure 3. LAT is linked to both hormone-sensitive prostate cancer (HSPC) and castration-resistant prostate cancer (CRPC). In untreated HSPC, 5-alpha reductase converts testosterone to dihydrotestosterone (DHT), which binds to the androgen receptor (AR), enters the nucleus, and stimulates LAT3 transcription, resulting in enhanced LAT3 expression and contributes to the mTORC1 pathway activation. When hormone therapy is used to treat PCa, testosterone levels fall, resulting in castration, and ARs that no longer bind DHT change, increase, and generate splicing variants. AR-V7, in particular, can enter the nucleus in the absence of testosterone activation, and 4F2hc is present in its downstream signaling. Furthermore, the removal of leucine from the cells results in the lack of eIF2 repression and the admission of ATF4 into the nucleus. It enhances LAT1 expression, and LAT1 and 4F2hc form a dimer, allowing leucine into the cell and promoting tumor cell proliferation [54].

3.2. LAT2

LAT2 is reported to be less distributed in malignant tumors except for neuroendocrine tumors [55] and more distributed in normal tissues [3]. Therefore, LAT2 may be used as a reference marker for normal benign tissue or as an indication of a good prognostic outcome. One study [56] examined LAT1-4 expression and its association with clinical outcomes in a combined cohort of more than 18,000 radical prostatectomy specimens. The expression of LAT1-3 in prostate cancer is higher than that in benign tissues except for LAT4. The expressions of LAT2, LAT3, and ASCT2 are negatively correlated with GS $\geq$ 8, lymph node invasion, and high Decipher score. The lowest decile of LAT3 and ASCT2 expression correlates with the worst MFS. LAT2 and LAT3 expression is associated with better clinical outcomes [56]. y + LAT2 (SLC7A6) is an alternative light subunit that constitutes the cationic and neutral amino acid heterodimer transport system y + L. A study [50] established the castration-resistant prostate cancer (CRPC) model LN-cr with androgen AR expression. Compared with LNCaP, y + LAT2 expression is increased in LN-cr. These results suggest that androgen removal induces the down-regulation of LAT3 and up-regulation of y + LAT2 in LNCaP cells.

3.3. LAT3

It is highly expressed in primary PCa [5]. Previous studies have shown that LAT3 expression is reduced in metastatic and/or castration-resistant cancers; therefore, LAT3 expression may be associated with androgen dependence in PCa [53]. In another word, LAT3 is highly expressed in prostate cancer cells that expressed the androgen receptor (AR). LAT3 is highly expressed in LNCaP and C4-2 cells that expressed AR but hardly expressed in PC3 and DU145 cells without AR. LAT3 mediates leucine uptake in LNCaP and PC3 cells [41]. Its expression is increased under the treatment of dihydrotestosterone and reduced under bicalutamide treatment [51]. Growth factors such as EGF activate the PI3K/Akt/mTORC1 signaling pathway, which regulates different protein synthesis programs leading to cell growth. This pathway relies on mTORC1 to detect adequate levels of intracellular amino acids, which inversely regulates LAT3 expression to enhance amino acid access [57]. LAT3 knockdown inhibits phosphorylation of mTOR, eukaryotic translation initiation factor 4EBP1, and ribosomal protein S6K1, but does not inhibit phosphorylation of Akt. And AR knockdown results are similar (Figure 3). Furthermore, as above mentioned, erythrogenesis involves increased uptake of neutral essential amino acids through LAT3 [40]. As red blood cells mature, their transcription profile changes, reflecting altered metabolic states, including induction of genes for iron and heme metabolism, as well as those involved in the amino acid cycle. The mRNA expression of LAT1 and LAT3 is significantly increased in mature red blood cells, but no 4F2hc expression is detected, suggesting that LAT1 may have no transport function [40]. The high expression of LAT3 in prostate cancer will undoubtedly promote the uptake of more amino acids by red blood cells on the other hand.

3.4. LAT4

There is currently little research on LAT4 and prostate cancer. A study [52] has shown that LAT4 expression is up-regulated in CRPC cell lines. Another study has found that 18F-labeled amino acids, such as 3-O-methyl-6-18F-fluoro-L-dopa (18F-OMFD) and 18F-fluorodihydroxyphenylalanine (18F-FDOPA), are important imaging agents for PET in vivo tumor display [58,59]. 18F-OMFD appears to be a suitable diagnostic imaging tracer for amino acid transport in poorly differentiated squamous cell head and neck carcinoma with increased LAT1 and LAT4 expression [59]. Similarly, 18F-OMFD and 18F-FDOPA should also have diagnostic values in CRPC with high LAT4 expression [52]. A study found that the expression of LAT4 increases after amino acid ingestion in mouse models treated with N-butyl- (4-hydroxybutyl) nitrosamine (BBN) [60]. However, further studies and evidence on the value of LAT4 in the diagnosis and treatment of prostate cancer are lacking.

Table 1 summarizes the specific relationship between LATs and prostate cancer, as well as the corresponding inhibitors (Table 1).

Table 1. The current relationship between LATs and prostate cancer. (Symbol '—' represents that this cell line does not express the corresponding LATs according to the citation paper).

LATs	PCa Cell Lines	Up-Regulation of Expression	Inhibitors	Be Inhibited Effects	Diagnosis/Treatment
LAT1 [23,33,41,43,50,53,61]	LNCAP	↑/—	T3, BCH, JPH 203, ESK242, SKN, OKY-034	Lower proliferation, Higher apoptosis, Lower leucine absorption, Lower mTORC1 activity, Amino acid stress, Reduced tumor metastasis ability.	Used as a PET tracer transporter in the diagnosis of malignant tumors. As a target for targeted therapy.
	C4-2	↑			
	PC3	↑			
	DU145	↑			
	VCAP	↑			
	22Rv1	↑			
LAT2 [55,56]	prostate specimen	↑	BCH	N/A	Associated with a better prognosis.
LAT3 [5,41,51,53,54,62,63]	LNCAP	↑	ESK242, ESK246	Lower proliferation, higher apoptosis, Reduced tumor metastasis ability.	As a tumor marker for HSPC to CRPC transformation, As a targeted therapeutic target.
	C4-2	↑			
	PC3	—			
	DU145	—			
LAT4 [42,52,58,59]	22Rv1 (Simulate the CRPC situation)	↑	N/A	Growth retardation in mouse models.	Possible as a PET tracer target for 18F-labeled amino acids in CRPC.

4. Prostate Cancer Diagnosis by LATs

4.1. LAT1

Among amino acid transporters, LAT1 is selectively hyperactive in a variety of cancer cells [10]. The pathway for enhancing LAT1-mRNA expression is not yet clear but includes carcinogenic Myc and hyposia-inducedfactors (HIFs) that can enhance its expression. That suggests the feedforward mechanism of tumor formation [64,65]. LAT1 can be used as a tumor marker for prostate cancer, and LAT1 expression is highly correlated with high proliferation index, stage, and poor prognosis [23,66].

In addition to being a tumor marker, LAT1 will play an important role in the diagnosis as a transporter. In other words, LAT1 selectively delivered drugs can be used for the diagnosis of PCa. LAT1 special use matrix of cancer diagnosis of positron emission computed tomography (PET) is a powerful technology for clinical prostate cancer detection. PET works by detecting the radioactive isotopes labeled on the tracer to find out where the tracer accumulates. Cancer cells accumulate PET tracers, which mimic the nutrients they need to proliferate. (18) F-labeled fluoroalkyl phenylalanine derivatives, as PET tracers, are more likely to bind to LAT1 in tumors, which demonstrates the effectiveness of 18f-labeled aromatic side chain pet tracer in the diagnosis of prostate malignancies [67]. The U.S. Food and Drug Administration approved trans-1-amino-3-18f-flucyclobutane carboxylic acid (anti-[18F]-FACBC) PET to detect prostate cancer in patients with elevated prostate-specific antigen after treatment in 2016 [68]. The utility of LAT1 in PET imaging has been demonstrated in clinical practice.

At present, Japan is further developing a new PET tracer based on LAT1 delivery, the NKO series. The goal is to adopt a simpler and more efficient 18f markup structure. At present, the clinical study in the normal human body has been completed [69].

4.2. LAT3

As mentioned above, the expression level of LAT3 is different in different stages of prostate cancer. Studies have shown that LAT3 is regulated by androgen receptors. LAT3 is significantly reduced in CRPC, and LAT3 is also reduced after androgen deprivation therapy. LAT3 is expected to be a tumor marker to judge the progression of prostate cancer from HSPC to CRPC [41,43,51,53].

4.3. LAT4

As mentioned above, 18F-OMFD has diagnostic value as a PET tracer in CRPCS with high LAT4 expression [59].

5. Inhibitors of LATs and Targeted Therapy of PCa

Inhibitors that target transporters include compounds that transport as a substrate and non-transport compounds that act as blockers or exert an inhibitory effect on isosteroids. As transport inhibitors, the current mainstream view of pharmacology believes that non-transport compounds are superior to transport compounds because non-transport compounds do not accumulate in cells and have high affinity [69]. 2-aminobicyclo-(2,2,2,1)-heptane-2-caryboxylic acid (BCH) (Figure 4(Aa)) is a nonmetabolic leucine analogue. BCH is a specific inhibitor of the sodium-independent L system (LAT1, LAT2, LAT3, and LAT4). In the study of LATs, BCH is widely used [53,70]. However, because BCH is delivered as a substrate and has a low specificity and affinity for LAT1, it has not been used at the clinical level. Clinical studies on anti-cancer drugs (inhibitors) targeting LATs mainly focus on LAT1 and LAT3.

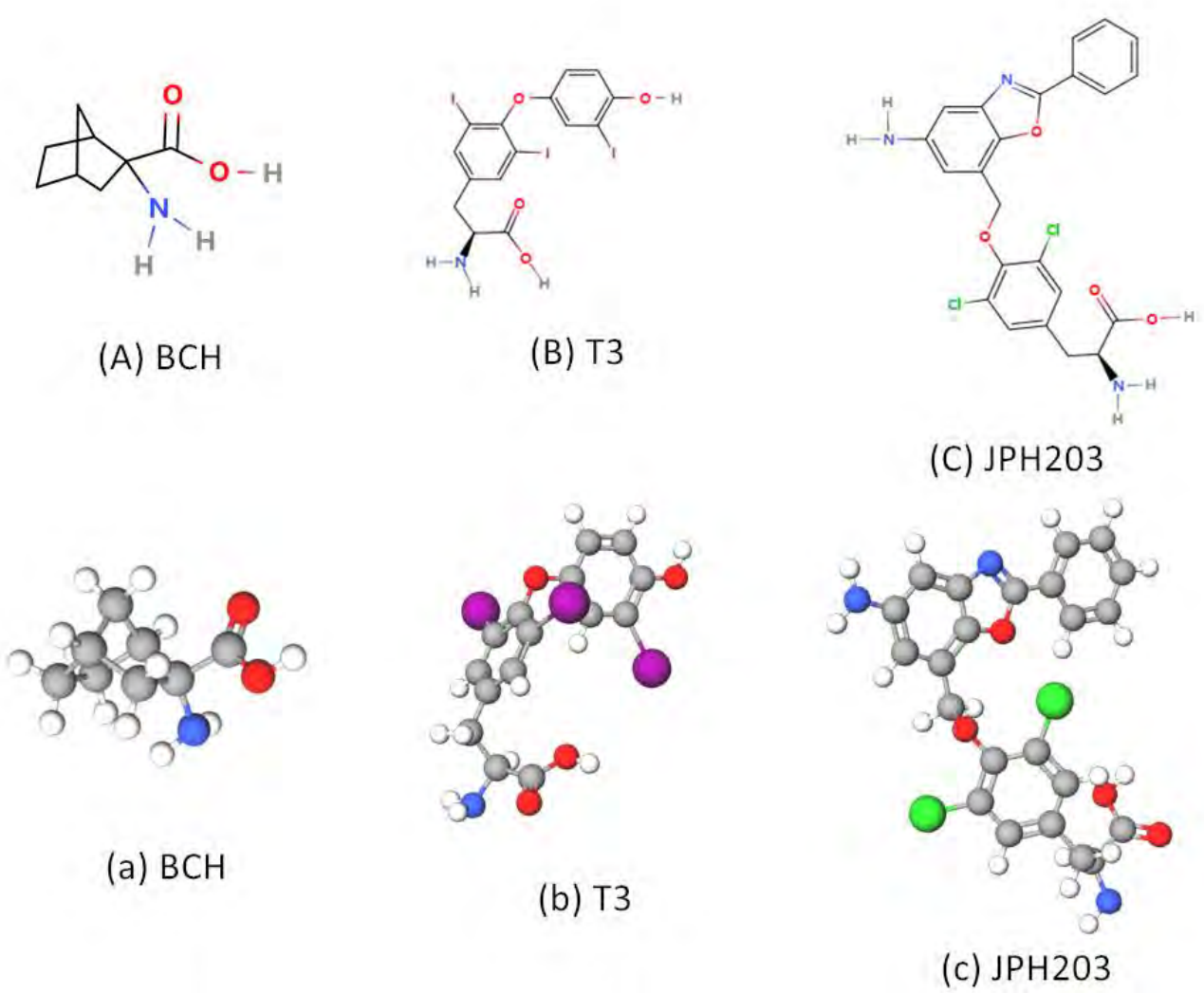

Figure 4. The 2D (**A**–**C**) and 3D (**a**–**c**) structures of LATs inhibitors. (**Aa**) BCH is a system L inhibitor. (**Bb**,**Cc**) The core structures of T3 and JPH203 both contain an amino acid backbone and a bulky side chain.

In LAT1, the earliest reported specific LAT1 inhibitor was the thyroid hormone triiodothyronine (T3) (Figure 4(Bb)), which showed a high inhibitory effect (low Ki value) and was almost non-transportable molecule [71], thus providing an idea for the research and development of LAT1 inhibitors. Therefore, JPH203 (KYT-0353) (Figure 4(Cc)) was successfully developed in 2009 as a non-delivery LAT1-specific inhibitor (LAT1 blocker) [72]. The core structures of T3 and JPH203 both contain an amino acid backbone and a bulky

and was almost non-transportable molecule [71], thus providing an idea for the research and development of LAT1 inhibitors. Therefore, JPH203 (KYT-0353) (Figure 4(Cc)) was successfully developed in 2009 as a non-delivery LAT1-specific inhibitor (LAT1 blocker) [72]. The core structures of T3 and JPH203 both contain an amino acid backbone and a bulky side chain (Figure 4(Bb,Cc). Then, JPH203 has recently been widely studied as a potent inhibitor of LAT1. JPH203, like T3, has a high affinity and is specific to LAT1, but does not affect LAT2. JPH203 interferes with the constitutive activation of mTORC1 and Akt, reduces c-MyC expression, and triggers a folding protein response mediated by CHOP transcription factors associated with cell death [73]. Since then, several studies have confirmed that JPH203 has a significant inhibitory effect on the growth of common tumor cells. A Phase I clinical study found that JPH203 is well tolerated and provided positive prognostic outcomes in the treatment of biliary tract cancer, with a disease control rate of approximately 60% against biliary tract cancer [74]. The authors are currently planning Phase I and II studies of JPH203 in CRPC. Although JPH203 has been shown in multiple studies to inhibit leucine uptake by tumor cells and has shown concentration-dependent cytotoxicity in vitro or performed well in transplanted tumor models, the human phase I clinical trial is a milestone. In addition, a Japanese research team has developed an SKN series of LAT1 inhibitors similar to T3 and JPH203 (Figure 5(Aa)) [75,76]. In recent years, a research team led by Professor Kanai at Osaka University is developing the OKY series of novel LAT1 inhibitors. Among OKY compounds, OKY-034 shows a high inhibitory effect and specificity against LAT1. The above amino acid LAT1 inhibitors are competitive inhibitors, but OKY-034 has a non-competitive inhibitor style because the structure of OKY-034 does not include the amino acid skeleton. The advantage of non-competitive inhibitors is that only small amounts (low concentrations) are required to show effect, due to the need to react competitively with the endogenous amino acid matrix. In addition, OKY-034 does not require large hydrophobic sites such as T3 and SKN, so it is relatively soluble and can be taken orally. Phase I/IIa trial of OKY-034 safety and efficacy in patients with pancreatic cancer is being conducted at Osaka University Hospital (UMIN000036395) [69]. It is believed that these drugs will soon be used to treat prostate cancer.

However, compared with LAT1, there is less evidence to support the general role of LAT2 in cancer, and it is more likely to be used as a benign biomarker. Since there is no known drug substrate or inhibitor targeting LAT2 except for BCH, unfortunately, no studies have been conducted on LAT2 as a tumor-targeted therapy. However, LAT2's innate ability to transport amino acids makes it a potential target for the diagnosis or treatment of prostate cancer based on amino acids.

In terms of LAT3, LAT3 knockdown inhibits cell proliferation, migration, invasion, and phosphorylation of p70S6K and 4EBP-1 [51]. Studies have shown that ESK242 and ESK246 are effective inhibitors of LAT3 (Figure 5(Bb)). ESK246 preferentially inhibits leucine transport through LAT3, while ESK242 can inhibit both LAT1 and LAT3. Its use in prostate cancer cells further suggests that ESK246 is a potent leucine uptake inhibitor that leads to decreased mTORC1 signaling, cyclin expression, and cell proliferation [63]. New anti-prostate cancer therapies targeting LAT3 may build on this.

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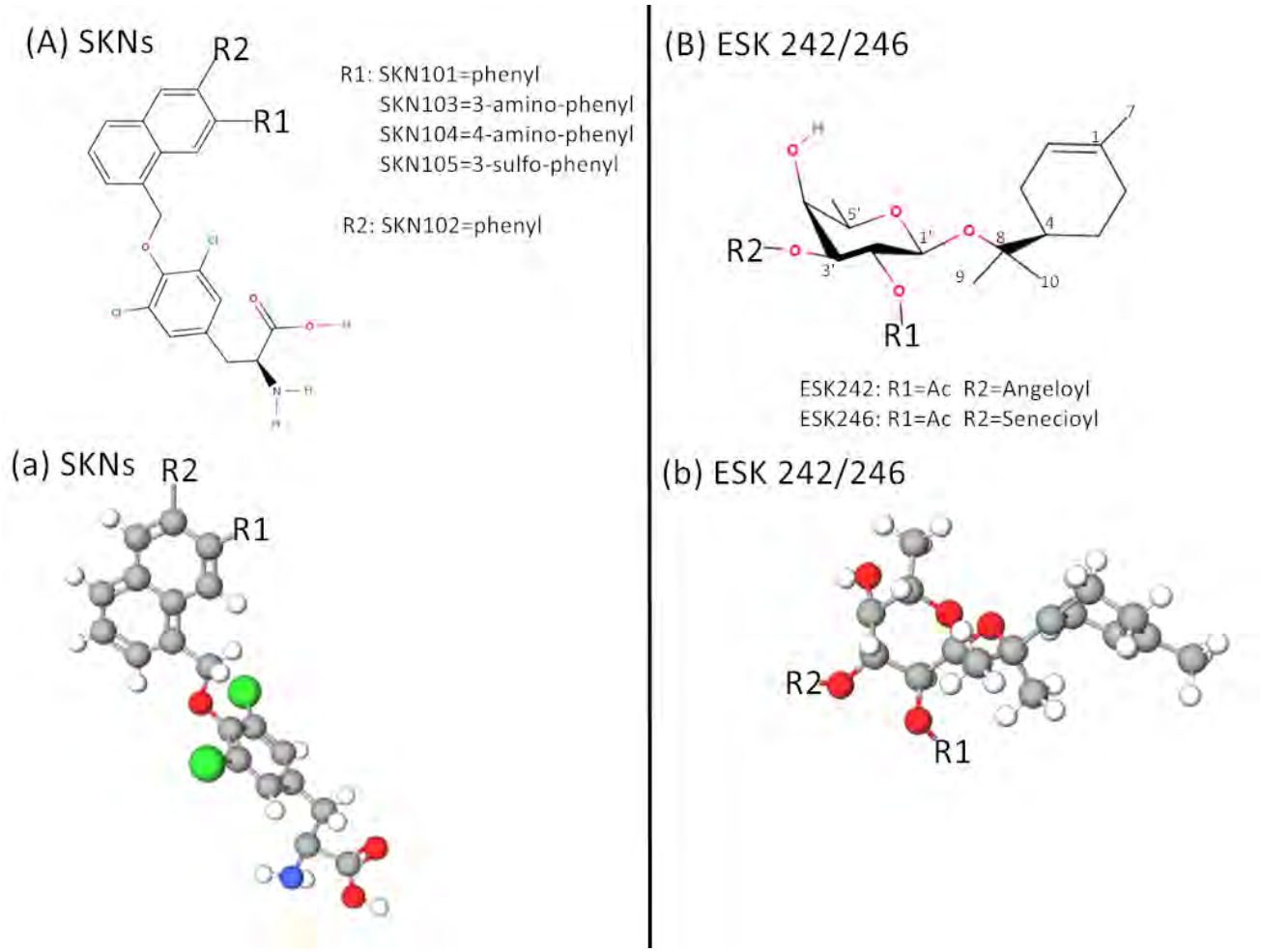

Figure 5. The 2D (**A**,**B**) and 3D (**a**,**b**) structures of LAT1 inhibitor SKN series and LAT3 inhibitor ESK series. (**Aa**) SKN series and JPH203 have similar molecular structures. (**B**,**b**) The ESK series shows a different molecular structure from LAT1 inhibitors such as T3, JPH203, and SKNs. ESK242 can inhibit both LAT1 and LAT3.

6. Conclusions

The L-type amino acid transport family has valuable clinical significance in malignant tumors represented by prostate cancer. LAT1 is not only a reliable tumor biomarker but also an imaging tracer target. LAT1 and LAT3 can be used in the diagnosis and prognosis of prostate cancer. Moreover, the specific expression levels of LAT1 and LAT3 have the value of judging the development of prostate cancer to castration-resistant prostate cancer, which can effectively guide the anti-androgen therapy of prostate cancer and predict the possibility of biochemical recurrence. In addition, LAT1 and LAT3 are also significant therapeutic targets for prostate cancer. Several inhibitors targeting the corresponding LATs are in clinical trials and are expected to be widely used in the targeted therapy of prostate cancer in the near future.

Author Contributions: X.Z. contributed to collecting bibliography, preparing figures, and writing; S.S. (Shinichi Sakamoto) contributed to drawing structure figures and writing; J.W., S.P., S.S. (Shinpei Saito), T.S. and Y.I. contributed to collecting bibliography; S.S. (Shinichi Sakamoto), N.A. and T.I. contributed to supervision of all the activities. The first draft of the manuscript was prepared by X.Z. and S.S. (Shinichi Sakamoto) revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The present study was supported by grants from Grant-in-Aid for Scientific Research (C) (20K09555 to S.S.), Grant-in-Aid for Scientific Research (B) (20H03813 to T.I.), and the Japan China Sasakawa Medical Fellowship (to X.Z.).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Abbreviation

PCa	prostate cancer
LAT	L-type amino acid transporter
mTOR	mammalian target of rapamycin
EAA	essential amino acid
SLC	solute vector family
TCR	T cell receptors
BCAA	branched-chain amino acid
mTORC	mammalian target of rapamycin complex
GAP	GTPase-activating protein
GATOR	GAP activity toward Rags
BCAT	branched-chain amino acid transferase
BCKA	branched-chain keto acid
Kyn	kynurenine
TDO	2, 3-Deoxygenase
IDO	indoleamine 2, 3-Dioxygenase
AHR	aryl hydrocarbon receptor
ADT	antiandrogen therapy
CRPC	castration-resistant prostate cancer
AR	androgen receptor
HSPC	hormone-sensitive prostate cancer
18F-OMFD	3-O-methyl-6-18F-fluoro-L-dopa
18F-FDOPA	18F-fluorodihydroxyphenylalanine
BBN	N-butyl- (4-hydroxybutyl) nitrosamine
HIFs	hyposia-inducedfactors
PET	positron emission computed tomography
BCH	2-aminobicyclo-(2,2,2,1)-heptane-2-caryboxylic acid

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Machine-learning predicts time-series prognosis factors in metastatic prostate cancer patients treated with androgen deprivation therapy

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Machine learning technology is expected to support diagnosis and prognosis prediction in medicine. We used machine learning to construct a new prognostic prediction model for prostate cancer patients based on longitudinal data obtained from age at diagnosis, peripheral blood and urine tests of 340 prostate cancer patients. Random survival forest (RSF) and survival tree were used for machine learning. In the time-series prognostic prediction model for metastatic prostate cancer patients, the RSF model showed better prediction accuracy than the conventional Cox proportional hazards model for almost all time periods of progression-free survival (PFS), overall survival (OS) and cancer-specific survival (CSS). Based on the RSF model, we created a clinically applicable prognostic prediction model using survival trees for OS and CSS by combining the values of lactate dehydrogenase (LDH) before starting treatment and alkaline phosphatase (ALP) at 120 days after treatment. Machine learning provides useful information for predicting the prognosis of metastatic prostate cancer prior to treatment intervention by considering the nonlinear and combined impacts of multiple features. The addition of data after the start of treatment would allow for more precise prognostic risk assessment of patients and would be beneficial for subsequent treatment selection.

Prostate cancer is one of the most common carcinomas, with an increasing incidence worldwide[1]. In Japan, prostate cancer was the leading cause of cancer and sixth leading cause of cancer-related deaths in 2016[2]. Deeper understanding of prostate cancer and the intrinsic function of androgens has led to the development of androgen deprivation therapy (ADT). ADT is the mainstay treatment for locally advanced and metastatic prostate cancer. ADT is also a treatment option for elderly patients with non-metastatic prostate cancer or those in poor general condition who are not candidates for surgery or radiation therapy. Prostate-specific antigen (PSA) is used as a prostate cancer-specific tumor marker that acts as a first guide and plays a key role in determining treatment efficacy of ADT. Recent reports have demonstrated that the modified Glasgow Prognostic Score (mGPS), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) levels, Eastern Cooperative Oncology Group (ECOG) performance status, and Gleason score are associated with different prognoses[3,4].

The prognosis of prostate cancer varies considerably depending on whether the disease is non-metastatic or metastatic[5]. Many prognostic studies on metastatic castration-resistant prostate cancer (mCRPC) have been reported, while less information is available on non-castrated metastatic prostate cancer (NCMPC). Among the few reports available, a prognostic prediction model was published by Glass et al. in 2003[6] that classified patients

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into three prognostic groups according to four risk factors: bone lesion localization, performance status, PSA, and Gleason score. Based on the model proposed by Glass et al., Gravis et al. proposed a prediction model[7] that is excellent in that it only uses a single feature, ALP, which is obtained in routine clinical practice. However, the performance of the prognostic prediction model is insufficient, with a concordance index (C-index) of 0.64. To further improve prediction accuracy, it would be necessary to consider the time variation and interaction of the factors used for the prediction[8].

Developments in computer technology have improved analytical methods for handling large-scale data, and machine learning has attracted attention also in the medical field. Machine-learning techniques are commonly used for data-driven diagnostic and prognostic predictions[9,10]. The greatest advantage of using machine learning is that it can be used to account for the combined, nonlinear effects of numerous variables and can make precise individualized predictions for heterogeneous patient populations. In recent years, machine learning-based survival analysis has been used in various carcinomas, handling many variables and enabling prognostic prediction with high accuracy[11–13]. In addition to cancer prognostic prediction, there are many other areas where machine learning can contribute to biomedical research, such as drug interaction analysis[14,15].

Therefore, the purpose of this study was to develop a clinically applicable prognostic prediction model for prostate cancer treated with androgen deprivation therapy based on multiple features using machine learning. We then additionally examined the impact on prediction accuracy of incorporating features after the start of treatment. To ensure applicability in clinical practice, this study used features obtained routinely in medical practice, such as peripheral blood sampling and urinalysis.

Result

Patient background. This study included 340 patients with prostate cancer. Of these, 30 patients who had started treatment at other hospitals were excluded (Fig. S1). A final total of 310 patients were included in the study, comprising 207 and 103 patients in the training and test cohorts, respectively. The median age was 74 years, and the median initial PSA level was 40.365. The rates of Gleason score ≥ 8 was 54.2%. The rate of metastasis was 41.6% (Table 1). No significant differences were observed between the training and test cohorts in patient backgrounds. Among the 36 features used as explanatory variables, only uric acid (UA) was significantly different between the training and test cohorts (Table 2).

Prognostic prediction at the start of treatment. To evaluate the usefulness of multiple variables for predicting prostate cancer prognosis, 36 features including age, peripheral blood tests, and urinalysis were used in the analysis. To maintain impartiality among models and avoid multicollinearity among features, the variables were first selected using RSF based on permutation importance calculated in the training cohort. Selected top important variables with positive permutation importance were used in subsequent RSF and Cox proportional hazards analyses. In addition, we created a prediction model for PSA (a tumor marker for prostate cancer) alone and compared its accuracy using the C-index (Fig. 1A). The C-indices for prediction in test cohort using the Cox proportional hazards model were 0.573, 0.488, and 0.582 for PFS, OS, and CSS, respectively. The corresponding C-indices for prediction using PSA alone were 0.684, 0.656, and 0.774, respectively. Finally, the corresponding mean C-indices (standard deviation) with RSF were 0.681 (0.002), 0.603 (0.005), and 0.832 (0.004), respectively. In terms of prediction at the start of treatment, the conventional prediction using PSA was almost as accurate as the RSF in predicting PFS, OS, and CSS, respectively. Next, we calculated the prognostic accuracy of the RSF model created above when applied separately to metastatic and non-metastatic prostate cancer patients. The results revealed improved OS prediction accuracy in metastatic prostate cancer, while, for non-metastatic tumors, predictive performance was poor for all predictions (Fig. 1B). We identified PSA as an important predictor in RSF for predicting PFS and LDH as an important predictor of OS and CSS (Fig. 1C–E).

Prognostic predictions considering temporal changes after the start of treatment. We further aimed to improve the prediction of metastatic prostate cancer by considering post-treatment changes. Patients with metastatic prostate cancer were assigned to the same training and test cohort as in the pretreatment analysis. In this analysis, the C-indices of the Cox proportional hazards model and prediction model using only PSA were calculated for comparison with the RSF model (Fig. 2). For predicting OS and CSS, the RSF model was more accurate than the other models: for the RSF model, it had the highest C-index (standard deviation) for predicting PFS at 150 days post-treatment at 0.766 (0.011), and at 120 days post-treatment the C-index for

Background	All patients (N = 310)	Training cohort (N = 207)	Test cohort (N = 103)	*P* value
Age, years (range)	74 (46–93)	74 (46–90)	74 (48–93)	0.2617
Initial PSA, ng/dL (range)	40.365 (0.19–13,050)	39.31 (2.05–13,050)	42.55 (0.19–6421.08)	0.6853
Gleason score ≥ 8, n (%)	168 (54.2)	116 (56)	52 (50.5)	0.3748
T ≥ 3, n (%)	193 (62.3)	128 (61.8)	65 (63.1)	0.8995
N+, n (%)	81 (26.1)	52 (25.1)	29 (28.2)	0.5848
M+, n (%)	129 (41.6)	87 (42)	42 (40.8)	0.9028

Table 1. Clinical backgrounds of 310 patients with prostate cancer. T ≥ 3 means tumor stage 3 or greater, N+ means lymph node metastasis, M+ means metastasis.

Factor	All patients (N = 310)	Training cohort (N = 207)	Test cohort (N = 103)	*P* value
Age (years)	74 (46–93)	74 (46–90)	74 (48–93)	0.2617
Initial PSA (ng/dL)	40.365 (0.19–13,050)	39.31 (2.05–13,050)	42.55 (0.19–6421.08)	0.6853
AST (U/L)	22 (11–129)	22 (12–95)	23 (11–129)	0.9234
ALT (U/L)	17 (5–102)	17 (5–102)	17 (5–80)	0.9014
LDH (U/L)	193.5 (82–4621)	197 (119–833)	192 (82–4621)	0.2807
GTP (U/L)	32 (9–358)	32.323 (10–215)	31.703 (9–358)	0.2882
TP (g/dL)	7 (5.1–8.6)	7 (5.1–8.4)	6.9 (5.1–8.6)	0.5483
Alb (g/dL)	4.1 (2.5–4.9)	4.1 (2.5–4.9)	4.1 (2.6–4.8)	0.9776
UA (mg/dL)	5.8 (2.2–12)	5.727 (2.2–9.2)	6 (3.4–12)	0.0414
UN (mg/dL)	16 (5–58)	16 (5–58)	16 (8–33)	0.4691
CRE (mg/dL)	0.84 (0.52–8.02)	0.84 (0.52–8.02)	0.84 (0.59–2.01)	0.417
Tbil (mg/dL)	0.7 (0.2–2.8)	0.7 (0.2–2.8)	0.7 (0.3–2.3)	0.4782
Dbil (mg/dL)	0.1 (0–0.3)	0.1 (0–0.3)	0.1 (0–0.3)	0.7917
TCHO (mg/dL)	186.2185 (101–303)	185 (101–275)	187.591 (119–303)	0.056
TG (mg/dL)	125.2795 (45–912)	121 (47–912)	136.711 (45–285)	0.8275
Ca (mg/dL)	9 (6.7–11.6)	9 (7.7–10.7)	8.9 (6.7–11.6)	0.6029
Na (mmol/L)	140 (130–146)	140 (130–146)	140 (132–144)	0.9252
K (mmol/L)	4.21845 (3.1–6.6)	4.2 (3.1–6.6)	4.3 (3.1–5.4)	0.2421
Cl (mmol/L)	106 (95–116)	106 (96–116)	105.693 (95–111)	0.8685
WBC ($\times 10^3/\mu L$)	6.2 (2.4–19.7)	6.2 (2.4–12.8)	6.3 (2.5–19.7)	0.4734
RBC ($\times 10^6/\mu L$)	4.33 (1.93–6.49)	4.35 (1.93–6.1)	4.29 (2.82–6.49)	0.9218
Hb (g/dL)	13.5 (5.5–18.3)	13.5 (5.5–17.6)	13.5 (8.1–18.3)	0.8833
HCT (%)	40 (16.5–53.8)	39.8 (16.5–51.9)	40.2 (24.3–53.8)	0.7497
MCV (fL)	92.4 (72.3–114)	92.3 (75.9–114)	92.9 (72.2–110)	0.6529
MCH (pg)	31.1 (22.5–38.1)	31.1 (24.6–37.5)	31.1 (22.5–38.1)	0.9092
MCHC (%)	33.6 (30.8–36.3)	33.6 (30.8–36.3)	33.6 (30.8–36.2)	0.4757
PLT ($\times 10^3/\mu L$)	206 (18–466)	205 (18–466)	211 (84–433)	0.5377
ALP (U/L)	247.5 (110–9481)	248 (110–9481)	246 (123–2469)	0.6496
PT (s)	11.4541 (9.9–20.4)	11.4022 (9.9–19.6)	11.66675 (10–20.4)	0.3152
PTINR	1.00371 (0.9–1.88)	1.00321 (0.9–1.76)	1.006855 (0.9–1.88)	0.6916
BS (mg/dL)	113.178 (74–282)	115.544 (74–282)	107.5 (86–213)	0.0984
CHE (U/L)	287 (112–539)	287 (115–539)	286.5 (112–468)	0.5798
CRP (mg/dL)	0.2 (0–24.9)	0.2 (0–24.9)	0.16444 (0–8.8)	0.1746
UpH	6 (5–8)	6 (5–8)	6 (5–8)	0.7532
URBC (/HPF)	1 (0–50)	1 (0–50)	1 (0–50)	0.7784
UWBC (/HPF)	1 (0–50)	1 (0–30)	1 (0–50)	0.2623

Table 2. Characteristics of analysis factor. The data in the brackets indicate a range of values.

predicting OS and CSS were 0.89 (0.006) and 0.883 (0.006), respectively. The Cox proportional hazards model and RSF had similar predictive performance in predicting PFS at 150 days after treatment initiation. On the other hand, the prediction performance of RSF was appreciably better than the other two models in predicting OS and CSS. Compared to the other prognostic prediction models, the RSF forecasting model tended to have less variation in forecast accuracy depending on the time of year. While RSF was able to predict prognosis for metastatic prostate cancer with relatively high accuracy, it was difficult to predict prognosis for non-metastatic prostate cancer with high accuracy (Fig. S2). In this prognostic analysis of metastatic prostate cancer patients, the addition of the Gleason score, an important pathologic factor in prostate cancer, as a predictor did not result in a notable improvement in prognostic accuracy (Fig. S3). The distribution of Gleason scores in patients with metastatic prostate cancer is shown in Table S1.

Permutation importance in RSF analysis. Feature importance can be used to explain the contribution of explanatory variables in machine learning predictions[16]. We used permutation importance, a type of feature importance, to evaluate the contribution of explanatory variables in the RSF. Permutation importance at the time of prediction when the C-index was maximum in each of the RSF analyses described above is presented in Fig. 2D–F. For PFS prediction at 150 days after the start of treatment, the most important variable was PSA after treatment. For the prediction of OS and CSS at 120 days after the start of treatment, the most important factors were LDH before treatment and ALP after treatment. For both OS and CSS prediction, PSA levels before and after treatment were not included as an important predictor.

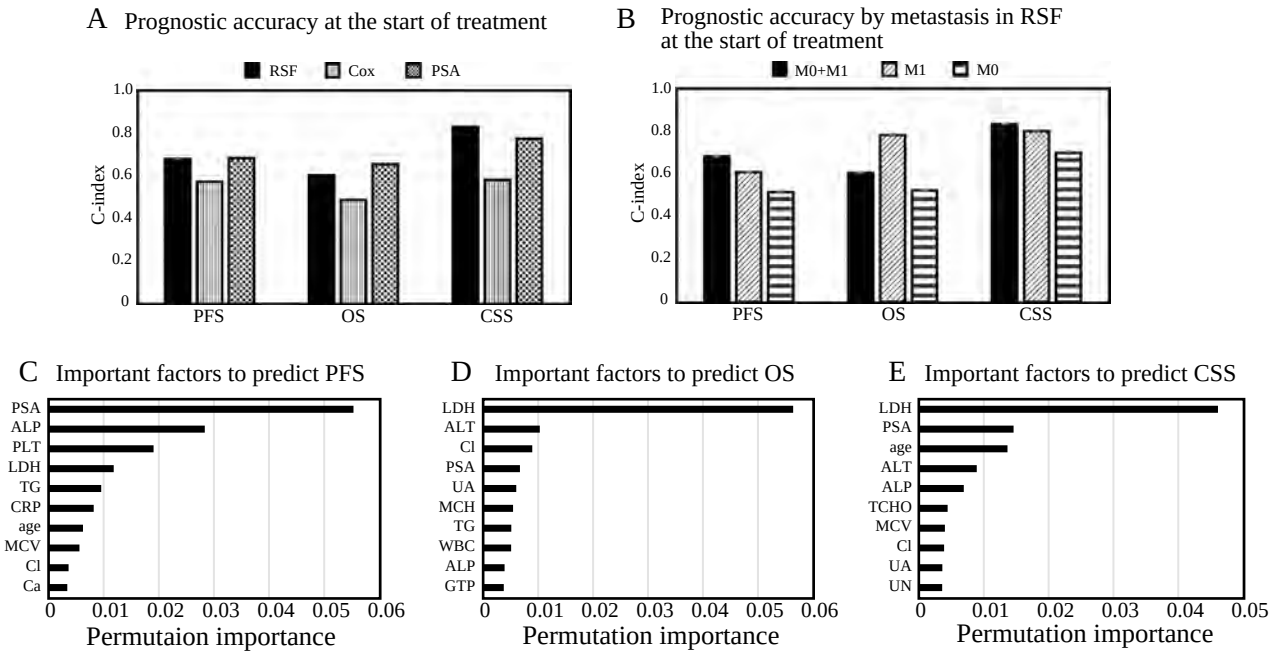

Figure 1. Comparison of accuracy of prognostic prediction models. (**A**) Comparison of C-index for each prognostic prediction model. Black, shaded, and horizontal bars indicate RSF, Cox proportional hazards, and PSA models, respectively. (**B**) Comparison of C-index for application of RSF model to patients with metastatic and non-metastatic prostate cancer. Black, striped, and dotted bars indicate all patients with prostate cancer, patients with metastatic prostate cancer, and patients with non-metastatic prostate cancer, respectively. (**C** to **E**) Permutation importance in prediction of progression (**C**), overall survival (**D**), and cancer specific survival (**E**) based on the RSF model.

Construction of survival trees based on RSF. As described in the previous section, prognosis prediction using RSF exhibited excellent accuracy. However, since RSF is an ensemble learning method with multiple survival trees and requires many explanatory variables, it is not easy to use it for prognostic prediction in real clinical practice. Therefore, we constructed a simplified survival tree model with a few most important variables in the RSF model. Since the contribution of post-treatment PSA was predominantly large in predicting PFS prognosis, and the benefit of combining multiple variables by survival tree was limited, we focused only on OS and CSS and constructed a survival tree model based on the top five important variables in the RSF models at 120 days after the start of treatment. The obtained survival trees predicting OS and CSS both consisted of LDH before treatment initiation and ALP 120 days after the start of treatment (Fig. 3A,C). The cut-off values of pre-treatment LDH and post-treatment ALP in the prediction models of OS and CSS were 248.5 IU/L and 342.5/326.5 U/L, respectively. The C-index for prediction accuracy was 0.85 for both OS and CSS. Based on these survival trees, three patient populations were identified that were associated with OS and CSS prognosis: the first was a very poor prognosis population with high preoperative LDH (> 248.5 IU/L), in which about 70% of patients would die within 5 years; the population with LDH < 248.5 IU/L was further divided into two groups based on post-treatment ALP. The group with high ALP is at intermediate risk and has a 5-year survival rate of about 70%. The population with low LDH before treatment and low ALP after treatment had a very good prognosis, with a 5-year survival rate exceeding 90% (Fig. 3B,D).

Discussion

Compared to conventional statistical analysis, machine learning can handle a large number and variety types of variables, and the machine can automatically learn and discover rules and patterns underlying the data. Various analyses using machine learning have been reported to improve the diagnostic rates of imaging and biopsy tests for prostate cancer[17,18]. However, prognostic analyses using machine learning for ADT remain scarce. In this study, we developed an approach to predict the prognosis of metastatic prostate cancer treatment over time: at the start of treatment and after the start of treatment. Pre-treatment and post-treatment features were combined to achieve a more accurate prediction.

We attempted to predict prognosis for both non-metastatic and metastatic prostate cancer, but it was difficult to predict prognosis in patients with non-metastatic prostate cancer (Fig. S2). The RSF model at the start of treatment showed improved predictive accuracy in metastatic prostate cancer patients, while it showed decreased accuracy in non-metastatic prostate cancer patients. This may be due to the fact that non-metastatic prostate cancer patients in this study had a smaller proportion of cancer deaths than metastatic prostate cancer patients, and included more senility and death from other causes, which are difficult to predict from clinical laboratory data. Moreover, prognostic factors for non-metastatic prostate cancer are limited, with only a few factors, such

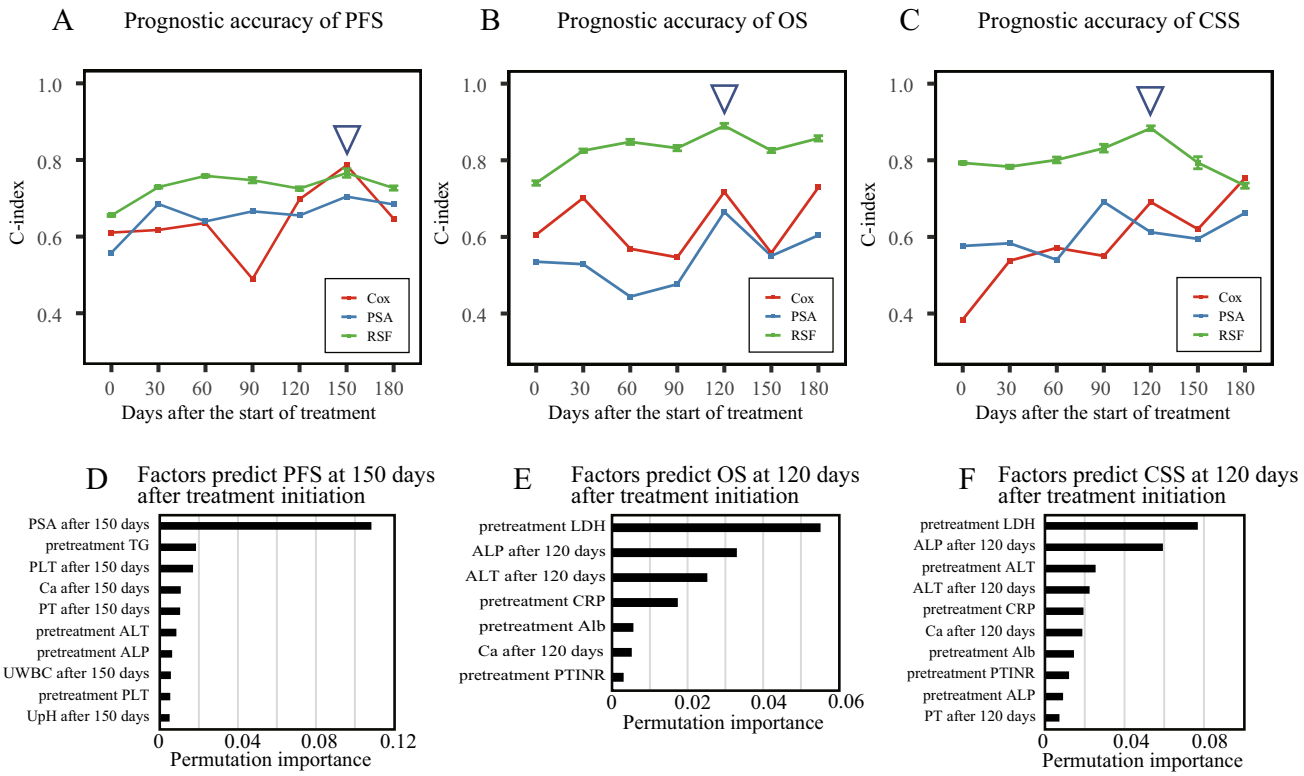

Figure 2. Time-series of prognostic accuracy for patients with metastatic prostate cancer. (**A** to **C**) Accuracy of prediction of progression (**A**), overall survival (**B**), and cancer specific survival (**C**). The green, red, and blue lines indicate the RSF, Cox proportional hazards, and prognostic PSA-based models, respectively. The triangle mark indicates the time point at which prediction accuracy was the highest for RSF prediction. Error bars represent standard deviations of 10 independent RSF. Permutation importance in prediction of progression at 150 days after treatment initiation (**D**), overall survival at 120 days after treatment initiation (**E**), and cancer-specific survival at 120 days after treatment initiation (**F**). The number of factors was defined as the top 10 factors or those with positive importance.

as PSA doubling time, reported in the literature[19–21]. Therefore, we focused on predicting the prognosis of metastatic prostate cancer. In this study, the RSF model was more accurate than other models in predicting OS and CSS in time-series metastatic prostate cancer. On the other hand, there was no significant difference in PFS prediction. First, the reason for the lack of significant difference in PFS prediction accuracy may be that factors other than PSA were less important in predicting PFS, since the definition of relapse in this study was biological relapse, which was defined as an increase in PSA. Second, the reason for the superior accuracy of the RSF model in predicting OS and CSS could be that parameters other than PSA are important as predictors in predicting OS and CSS, as shown by the results of Permutation Importance. Furthermore, regarding the difference between the RSF model and the Cox proportional hazards model, the RSF model may have been able to make more accurate predictions for many parameters in terms of its ability to make nonlinear predictions. However, since over-fitting should also be considered in this respect, we believe that validation using external data will be necessary in the future. Regarding the tumor marker PSA, our previous study reported no difference in OS according to initial PSA levels in patients with metastatic prostate cancer[22]. For prognostic factors other than PSA, the modified Glasgow Prognostic Score (mGPS), Eastern Cooperative Oncology Group (ECOG) performance status, LDH, ALP, and Gleason Score have been reported as prognostic factors for metastatic prostate cancer[3,4]. Among Japanese patients with de novo metastatic prostate cancer, LDH and C-reactive protein (CRP) have been reported as independent risk factors for OS in analyses identifying true high-risk groups that meet the CHAARTED or LATITUDE criteria[23]. Several studies support the results of this study. However, these were all prognostic analysis based on data at the start of treatment and did not include post-treatment changes. While prognostic predictions based on data at the start of treatment are important, the course of treatment affects the prognosis, and in some cases the actual prognosis differs from the initial risk assessment. To identify such cases and enable a more accurate prognosis, it is necessary to add post-treatment data as predictors and to update the prediction. In this study, we could first identify the poor prognosis group based on LDH at the start of treatment for both OS and CSS, and further classified the remaining patients into two groups with different prognoses using ALP after the start of treatment. This suggests that additional risk assessment during the course of treatment, in addition to risk classification at the start of treatment, can provide a more accurate prognosis. From a pathological perspective, we used the Gleason score in the RSF analysis, which has been used in existing risk classifications, but this did not clearly improve the C-index. Patients with metastatic prostate cancer tend to have high Gleason scores, and in fact, Gleason score ≥ 8 accounted for more than 70% of the patients in this case group.

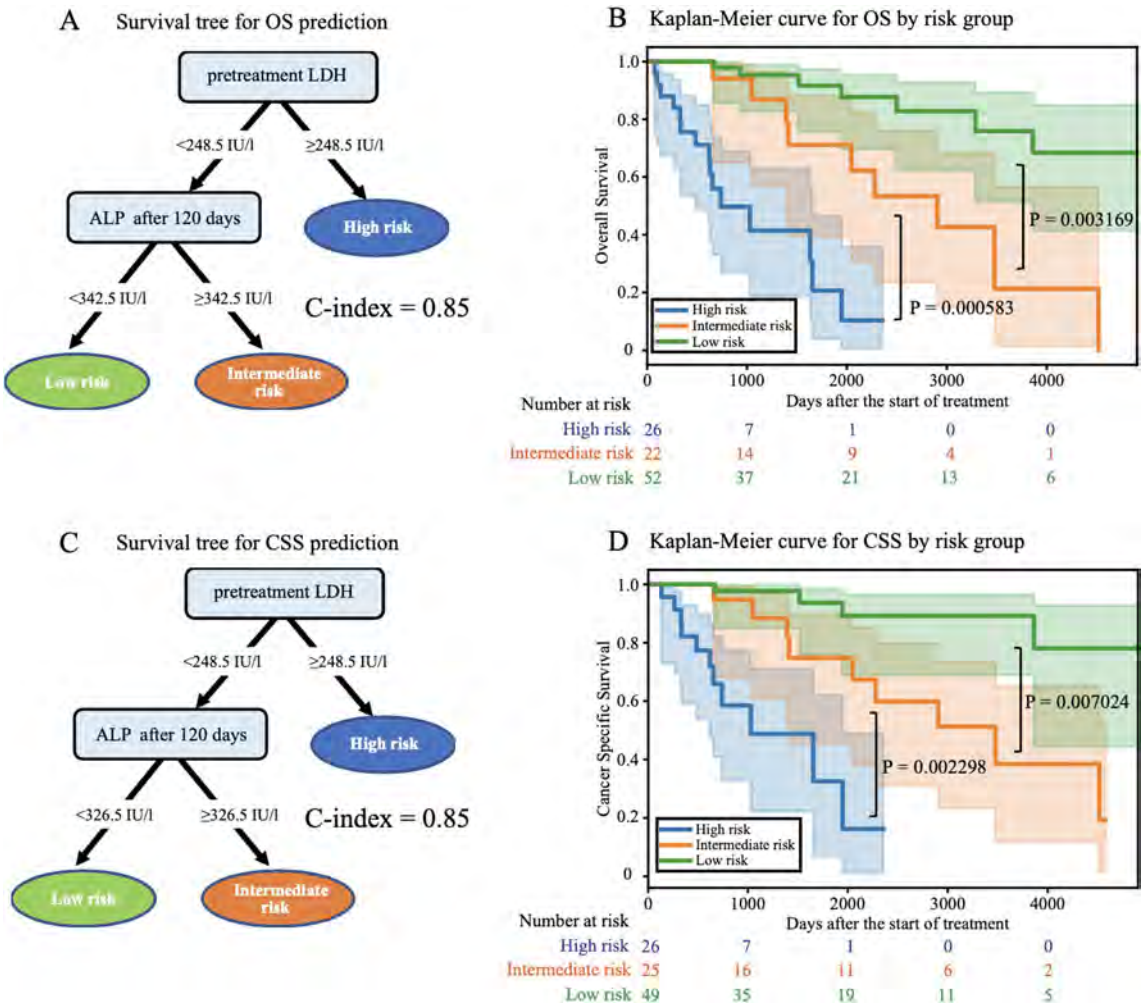

Figure 3. Survival tree predicting overall survival (**A**) and cancer-specific survival (**C**). Kaplan–Meier curves of survival tree prognostic classification results for overall survival prediction (**B**) and cancer-specific survival prediction (**D**). P-values were calculated by the log-rank test.

Gravis et al. reported a prediction model for NCMPC based on the prediction model proposed by Glass et al.[7]. They claimed that ALP levels at the start of treatment (normal vs. abnormal) were the strongest predictor of OS. This prediction model had a C-index of 0.64, was simpler than the prediction model developed by Glass et al., and exhibited comparable performance. The C-index of the model reported by Gravis et al. was 0.72 in the analysis using the data in this study. The C-index for our RSF model in this study using the data at the start of treatment was 0.74. Although our RSF model was only slightly more accurate than the previously reported model, the C-index was improved to 0.85 in this study by creating an algorithm using a survival tree with the addition of time-series data. The new algorithm for metastatic prostate cancer we have created based on the survival tree made predictions using two variables (pre-treatment LDH and post-treatment ALP) with a C-index of 0.85, which was higher than the accuracy of previous prediction models. LDH and ALP values can be obtained from routine blood tests and can be used for time-series evaluation.

Our study had several limitations. First, it was a retrospective analysis with a limited number of cases at a single institution, and there may have been a selective bias. In general, machine learning methods divide datasets into training and test data, create a prediction model with the training data, and evaluate the model using the test data. If the number of cases is small, a biased prediction model (overfitting) may be created if the training data have extreme characteristics. We used data from 129 patients with metastatic prostate cancer for the training in our analysis. To increase variation in the training data and suppress overfitting, we intend to conduct further analysis using larger-scale data from multiple institutions in the future. In this study, we performed random data splitting. Although there were no significant differences between the train cohort and the test cohort, it is necessary to consider the use of data splitting methods such as cross validation in future analyses to create a new model. Second, because we defined progression as biological progression caused by elevated PSA levels, post-treatment PSA inevitably became the most important factor for predicting progression. Future research should focus on clinical progression, such as disease worsening on imaging and the appearance of new metastases.

In conclusion, this study demonstrated that machine learning and combined assessment of pre- and post-treatment variables were useful for creating an accurate prognostic prediction model for ADT in metastatic

prostate cancer. This result may be harnessed as a new evaluation index for the treatment of metastatic prostate cancer.

Methods

Patient selection and analysis factors. This retrospective study included 340 patients with prostate cancer who received ADT as an initial treatment between 1996 and 2019 at the Department of Urology, Chiba University Hospital. Of these, 30 patients who had started treatment at other hospitals were excluded. The dataset was randomly divided into training and test cohorts. In total, 207 and 103 patients were classified into the training and test cohorts, respectively. We first analysed 36 features before treatment including age at diagnosis, peripheral blood sampling, and urinalysis to examine their association with progression-free survival (PFS), overall survival (OS), and cancer-specific survival (CSS). An additional analysis focusing on patients with metastatic prostate cancer was performed, which considered data at the start of treatment as well as subsequent changes. In the analysis, 35 features after the start of treatment including peripheral blood sampling and urinalysis were combined with the 36 pretreatment features and used for prediction. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. This retrospective study of clinical information was approved by the Ethics Committee of Chiba University (Institutional Review Board (IRB) no. M10238). The IRB waived the requirement for written consent in this study due to the retrospective nature of data collection.

Survival analysis. We employed random survival forests (RSF) for machine-learning survival analysis. The rationale for this is as follows. First, Random forests and derivatives outperform other machine learning methods in predictions using clinical laboratory values[24,25]. Secondly, RSF is implemented within scikit-survival, making it easy to calculate variable importance and transfer it to the survival tree model, which is also implemented in scikit-survival. Finally, like random forests, RSFs are suitable for variable selection because they selectively use a small number of variables[26]. RSF is a nonlinear survival model that combines ensemble learning and decision tree[27]. In RSF, multiple sets of data termed bootstrap samples are created. At each node of the survival tree, feature and its threshold value were determined such that the difference in hazard function between cases separated by the nodes was maximized. The ensemble hazard function of each patient was estimated by averaging the hazard functions of multiple trees created in this manner. In this study, RSF was used to predict PFS, OS, and CSS. Analysis was performed using scikit-survival Python package. We ran sksurv.ensemble.RandomSurvivalForest with the default parameters, except for the following parameters; n_estimators = 2000, min_samples_split = 10, min_samples_leaf = 15. The reason for using nearly default parameters is that hyperparameter optimization under limited training data conditions may result in lower accuracy, and random forests are robust to hyperparameter changes[28]. Since RSF uses bootstrap samples, the value of the estimated survival function varies slightly with each run. Therefore, we ran the RSF 10 times independently and used the average C-index as the prognostic performance indicator. We calculated permutation importance to evaluate the contribution of explanatory variables to RSF prediction performance. The permutation importance indicates the change in predictive performance (AUC in this case) when an explanatory variable is randomly shuffled, with a positive importance indicating that the variable is necessary for prediction and a negative importance indicating that using the variable reduces predictive performance[29]. For example, if the AUC drops by 0.05 when a variable is randomly shuffled, the permutation importance score for that variable is 0.05. Permutation importance was calculated using eli5 Python package.

A Cox proportional hazards model was used as the conventional statistical survival analysis for comparison. To make the conditions fair across models, the variables were selected based on the permutation importance calculated by RSF pretraining, and the same variables were used in the Cox proportional hazards model.

Survival tree. A survival tree represents the individual tree comprising the aforementioned RSF. This method analyses data using a tree diagram and exhibits excellent semantic interpretability in that it visualizes the classification criteria, facilitating comprehension of the results[30]. RSF can calculate feature importance during classification. By integrating the results of multiple survival trees, RSF allows highly accurate predictions for individual patients, but make it difficult for humans to interpret the predictive results and rationale. In this regard, survival tree may be a better solution for clinical implementation. In this study, we developed survival trees for OS and CSS using the top five important features obtained in the RSF analysis. We used the Optuna Python package to optimize the parameters of survival tree to achieve the highest prediction rate in training cohort[31].

Missing value imputation. To compensate for missing values in the dataset used in this study, we used the missForest algorithm implemented in R[32]. MissForest is a non-parametric imputation method that uses a random forest which can learn nonlinear relationship between variables, easily handle mixed-type data, and calculate out-of-bag (OOB) errors. First, the average value was used to tentatively fill the missing values, and the random forest was then repeatedly applied to predict the missing parts. Stekhoven et al. reported that missForest was superior to other widely used imputation algorithms such as KNNimpute, MICE, and MissPALasso.

Evaluation of survival model accuracy. The predictive performance of the survival models, including RSF, Cox proportional hazards model, and survival tree, was evaluated using the Harrell's concordance index (C-index). The C-index is a generalization of the area under the ROC curve (AUC) that considers censored data[33]. This represents an assessment of the discriminatory power of the model, which is the ability of the model to correctly provide a ranking of survival times for each patient based on hazard function. Time-dependent

ROC analysis is another method of evaluating prediction accuracy in survival analysis. However, we adopted the C-index to express the transition of prediction accuracy in the time-series analysis in an easily understandable manner, given the need for analysis at multiple time points after the start of treatment.

Statistical analysis. The Kaplan–Meier method was used to generate survival curves to evaluate survival probability of given groups. Statistical difference in the survival probabilities between groups was assessed using log-rank test. For the analysis of the training and test cohorts, Welch's t-test and Fisher's exact test were used for continuous and categorical variables, respectively. Statistical analysis was performed using JMP® 15.2. The significance level for each test was set at $\alpha = 0.05$.

Data availability

The datasets generated and analysed during the current study are not publicly available due to ethical regulations because the data contain personal information but are available from the corresponding author on reasonable request.

Received: 17 January 2023; Accepted: 5 April 2023
Published online: 18 April 2023

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Acknowledgements

We thank the members of the Urology Department of Chiba University School of Medicine for their enthusiastic clinical practice and the Artificial Intelligence Medicine Department of Chiba University Graduate School of Medicine for technical assistance. This work was supported by a Grant-in-Aid for Scientific Research (C) (grant #20K09555) to SS, Grant-in-Aid for Scientific Research (B) (grant #20H03813) to TI, Grant-in-Aid for Scientific Research (B) (grant #21H03065) to NA and Japan Science and Technology Agency (JST) CREST Grant (grant #JPMJCR20H4) to EK.

Author contributions

S.S. participated in study design, conduct of the study, data collection, data analysis, and writing of the article; S.S. participated in study design and revision of the article; K.H. and K.S. participated in study design, data collection, and data analysis; X.Z., K.W., M.K., S.K., N.T., T.S. and Y.I. participated in data collection; N.A and T.I. participated in the study design and revision of the article; E.K. participated in the study design, data analysis, and revision of the article.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-023-32987-6.

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EUROPEAN UROLOGY ONCOLOGY xxx (xxxx) xxx–xxx

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The Oncological and Functional Prognostic Value of Unconventional Histology of Prostate Cancer in Localized Disease Treated with Robotic Radical Prostatectomy: An International Multicenter 5-Year Cohort Study

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Article info

Article history:
Received 2 October 2023
Received in Revised form
3 December 2023
Accepted 11 December 2023

Associate Editor:
Guillaume Ploussard

Keywords:
Prostate cancer
Cribiform pattern
Intraductal prostate cancer
Ductal prostate cancer
Unusual histologies

Abstract

Background and objective: The impact of prostate cancer of unconventional histology (UH) on oncological and functional outcomes after robot-assisted radical prostatectomy (RARP) and adjuvant radiotherapy (aRT) receipt is unclear. We compared the impact of cribriform pattern (CP), ductal adenocarcinoma (DAC), and intraductal carcinoma (IDC) in comparison to pure adenocarcinoma (AC) on short- to mid-term oncological and functional results and receipt of aRT after RARP.

Methods: We retrospectively collected data for a large international cohort of men with localized prostate cancer treated with RARP between 2016 and 2020. The primary outcomes were biochemical recurrence (BCR)-free survival, erectile and continence function. aRT receipt was a secondary outcome. Kaplan-Meier survival and Cox regression analyses were performed.

Key findings and limitations: A total of 3935 patients were included. At median follow-up of 2.8 yr, the rates for BCR incidence (AC 10.7% vs IDC 17%; $p < 0.001$) and aRT receipt (AC 4.5% vs DAC 6.3% [$p = 0.003$] vs IDC 11.2% [$p < 0.001$]) were higher with UH. The 5-yr BCR-free survival rate was significantly poorer for UH groups, with hazard ratios of 1.67 (95% confidence interval [CI] 1.16–2.40; $p = 0.005$) for DAC, 5.22 (95% CI 3.41–8.01; $p < 0.001$) for IDC, and 3.45 (95% CI 2.29–5.20; $p < 0.001$) for CP in comparison to AC. Logistic regression analysis revealed that the presence of UH doubled the risk of new-onset erectile dysfunction at 1 yr, in comparison to AC (grade group 1-3), with hazard

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https://doi.org/10.1016/j.euo.2023.12.006

ratios of 2.13 ($p < 0.001$) for DAC, 2.14 ($p < 0.001$) for IDC, and 2.01 ($p = 0.011$) for CP. Moreover, CP, but not IDC or DAC, was associated with a significantly higher risk of incontinence (odds ratio 1.97; $p < 0.001$). The study is limited by the lack of central histopathological review and relatively short follow-up.

Conclusions and clinical implications: In a large cohort, UH presence was associated with worse short- to mid-term oncological outcomes after RARP. IDC independently predicted a higher rate of aRT receipt. At 1-yr follow-up after RP, patients with UH had three times higher risk of erectile dysfunction post RARP; CP was associated with a twofold higher incontinence rate.

Patient summary: Among patients with prostate cancer who undergo robot-assisted surgery to remove the prostate, those with less common types of prostate cancer have worse results for cancer control, erection, and urinary continence and a higher probability of receiving additional radiotherapy after surgery.

1. Introduction

Prostate cancer (PCa) is the second most frequently diagnosed cancer among men worldwide [1], with a worldwide estimated incidence of 1 414 259 new cases in 2020 [2]. PCa is a urological malignancy for which the economic burden is growing, especially for the elderly population [3]. Acinar adenocarcinoma (AC) is the most prevalent PCa histology. The Gleason grade, and the International Society of Urological Pathology (ISUP) grade group (GG) derived from the Gleason system [4], is one of the most important prognostic factors and is widely used for driving disease management plans [5]. In addition to the Gleason score, a recent systematic review demonstrated that the presence of an unconventional histology (UH), in particular intraductal carcinoma (IDC), cribriform pattern (CP), or ductal adenocarcinoma (DAC), may be associated with worse oncological prognosis in comparison to conventional and mucinous or prostatic intraepithelial neoplasia (PIN)-like PCa [6]. While the histology results in the review were for prostate biopsy and radical prostatectomy (RP) specimens, recent studies have suggested that prostatectomy rather than biopsy should be the gold standard in determining Gleason scores and diagnosing IDC/CP, in light of the limited concordance rates [7,8]. Both the Genitourinary Pathology Society and the ISUP recommend reporting of the percentage of Gleason pattern 4 and the presence of CP (present in 1% of PCa cases), which is associated with higher rates of biochemical recurrence (BCR; hazard ratio [HR] 2.1) and cancer-specific mortality (HR 3.3) [9–12]. According to the literature, DAC is the second most frequent UH and its presence predicted prostate-specific antigen (PSA) recurrence in one study [13] and was associated with worse overall mortality and metastasis-free survival [14]; IDC was found to be more prevalent in metastatic PCa [15]. However, most of these data were collected in the early 2000s, details regarding the surgical approaches used were lacking, and the UH impact on functional outcomes was not explained.

RP is one of the curative treatment options for localized PCa [5]. In the past decade, the techniques for RP have evolved from open to minimally invasive surgery. And robot-assisted laparoscopic RP (RARP) has become an established and safe surgical modality [16]. Unanswered questions remain regarding the impact of UH in PCa on functional and oncological results after RARP.

Although current guidelines support the use of adjuvant radiation therapy (aRT) in patients with pN0 status with GG 4–5 pT3 disease ± positive margins [5], there is no clear recommendation on the need for adjuvant treatment for patients with CP, DAC, or IDC types.

The aim of this study was to provide contemporary updates on the prognostic value of PCa UH on oncological and functional outcomes, by investigating a large multicenter cohort of patients treated with RARP. The secondary aim was to evaluate potential differences in the rates of aRT receipt after RARP among the various UH groups.

2. Patients and methods

We retrospectively collected data for the consecutive RARP cases performed from 2016 to 2020 in seven international high-volume centers. Patients with prior PCa treatment and mixed histology subtypes were excluded. Preoperative imaging for metastatic screening was performed for patients with intermediate- or high-risk cancer according to the European Association of Urology (EAU) risk categories. Decisions on the need for lymph node dissection were made according to risk nomograms.

Baseline demographic (age, PSA, and prostate size) and pathological data (histological patterns, tumor and node stages) were retrieved and analyzed.

2.1. Outcomes of interest

The oncological outcomes of interest included BCR, defined according to EAU guidelines as two consecutive rising PSA values >0.2 ng/ml [5] and aRT receipt, defined as RT planned after RARP on the basis of clinicopathological risk factors before the occurrence of BCR and performed within 4–22 wk after RARP [17], regardless of dose and fractionation. Data for other oncological outcomes, such as the incidence of positive surgical margins, lymph node involvement, and nodal and distant metastases, were also collected.

The functional outcomes of interest included continence, defined as no more than one protective pad per day [18], and potency, defined as the ability to obtain an erection

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rigid enough for intercourse with or without the use of a phosphodiesterase type 5 inhibitor at least half of the time [19].

2.2. Pathological evaluation and study groups

In each centre, RARP specimens had been sampled and embedded for diagnostic purposes as previously described [20] and were assessed by dedicated uropathologists. Data on histological types, Gleason score, ISUP grade group according to the 2014 ISUP/2016 World health Organization (WHO) guidelines [21], presence of IDC, pT and pN stage according to the 8th edition of the American Joint Committee on Cancer TNM scheme [22], and surgical margin status were retrospectively retrieved from reports for RARP specimens.

Four groups of prostate malignancy were considered: pure AC, AC with CP, DAC, and IDC (Fig. 1A). The latter three groups consisted of malignant prostatic lesions with CP and were defined according to the WHO classification [23]. In brief, DAC is composed of papillary structures and/or cribriform glands lined by tall columnar pseudostratified cells; basal cells are absent. We considered both the pure form and DAC admixed with acinar AC. IDC was defined as complex cribriform growth and lumen expansile proliferation of malignant epithelial cells within native ducts and acini with intact basal cells. We included IDC cases associated with invasive acinar AC. CP, one of the four patterns of Gleason grade 4 AC, was defined as a confluent sheet of contiguous malignant epithelial cells with multiple glandular lumina without intervening stroma or mucin separating the glandular structures [9].

2.3. Statistical analyses

Statistical comparisons were made among the four groups. Results for continuous variables are presented as the median and interquartile range (IQR) and were compared using a Mann-Whitney *U* test. Results for categorical variables are presented as the frequency and percentage and were compared using a χ^2 test or Fisher's exact test. Kaplan-Meier curves were used to compare outcomes, and Cox regression analysis was performed to adjust for potential confounding factors. SPSS was used for the statistical analyses. Given that DAC without an AC component is, by definition, assigned a Gleason score of 4 + 4 = 8 (ISUP GG 4), for their similar clinical behavior [24], we compared AC GG 1–3 versus GG 4 versus GG 5 versus the three UH types.

3. Results

Among a total of 5005 patients, 1070 had mixed or unreported histology and were therefore excluded, leaving 3935 PCa cases suitable for analysis. Among these, 3126 patients had pure AC, 174 had AC with CP, 447 had DAC, and 188 had IDC (Fig. 1A).

3.1. Baseline characteristics

Baseline patient and disease characteristics are listed in Table 1. Overall, the median age was 65 yr (IQR 60–70), the median PSA was 6.8 ng/ml (IQR 5–10), and the median prostate size was 38 cm^3 (IQR 28–53). Regarding the ISUP grade groups in the overall cohort, 22.6% had GG 1, 38.0% had GG 2, 17.6% had GG 3, 7.4% had GG 4, and 5.6% had GG 5 disease; the ISUP grade group was not reported for 8.7% of the cohort.

The IDC and CP groups had similar median PSA and prostate size to the AC group. The DAC group had significantly lower median PSA (6.1 ng/ml) than the AC group (6.9 ng/ml) but similar median age and prostate size. In comparison to the AC group, ISUP grade groups were significantly higher for each of the UH groups ($p < 0.001$). In the AC group, 77.6% of cases were GG 1–3 disease, whereas the majority of cases in the UH groups were GG 2–3 disease (DAC 66.7%, IDC 74.5%, CP 81.6%).

3.2. Oncological outcomes

At median follow-up of 2.8 yr, BCR was more frequent for IDC than for AC (17% vs 10.7%; $p < 0.001$). The rate of positive surgical margins was significantly higher in DAC (33.6%; $p = 0.04$), IDC (42.6%; $p < 0.001$), and CP (43.1%; $p < 0.001$) than in AC (27.3%). Lymph node involvement was also more common in DAC (6.9%; $p < 0.001$) and IDC (8%; $p < 0.001$) than in AC (0.5%). More nodal recurrence was observed in DAC (1.6%; $p < 0.001$) and CP (2.9%; $p = 0.049$) than in AC (0.4%). Distant metastases occurred more frequently in DAC (1.1%; $p = 0.002$) and IDC (3.7%; $p = 0.002$) than in AC (0.9%; Table 2).

Kaplan-Meier curve revealed significant differences in 5-yr BCR-free survival (Fig. 1B). In comparison to AC GG 1–3, IDC had the worst BCR rate, followed by CP, AC GG 5, DAC, and AC GG 4 ($p < 0.001$).

Univariable Cox regression analysis revealed significantly poorer 5-yr BCR-free survival for the UH groups in comparison to pure AC, with HRs of 1.67 for DAC, 5.22 for IDC, and 3.45 for CP (Table 3). According to multivariable Cox regression analysis with AC GG1–3 taken as the reference, the significant predictors for BCR at 5 yr included DAC (HR 3.15; $p < 0.001$), IDC (HR 5.63; $p < 0.01$), CP (HR 3.94; $p < 0.001$), PSA (HR 1.63; $p = 0.001$), and pT3b stage (HR 2.19; $p = 0.007$; Table 4). Meanwhile, GG 4 (HR 2.07; $p = 0.096$), GG 5 (HR 3.15; $p = 0.101$), age (HR 1.0; $p = 0.98$), pT3a (HR 2.19; $p = 0.097$), positive margin status (HR 1.47; $p = 0.069$), and positive node status (HR 0.88; $p = 0.724$) were not significant predictors for BCR.

3.3. Functional outcomes

In comparison to AC, a two-to-three-fold increase in the risk of de novo erectile dysfunction at 1 yr after RARP was observed for each UH subgroup. In addition, CP was associated with twofold higher risk of incontinence than AC at one yr after RARP (Table 5). Upon multivariable Cox regression analysis taking AC GG 1–3 as the reference, the presence of DAC (HR 2.13; $p < 0.001$), IDC (HR 2.14; $p < 0.001$), or CP (HR 2.01; $p = 0.011$) doubled the risk of erectile dysfunction at one year postoperatively (Table 6). The other significant predictors for erectile dysfunction found were pT3a stage (HR 1.67; $p < 0.001$) and pT3b stage (HR 1.69; $p = 0.003$). GG 4 disease, GG 5 disease, and positive margin

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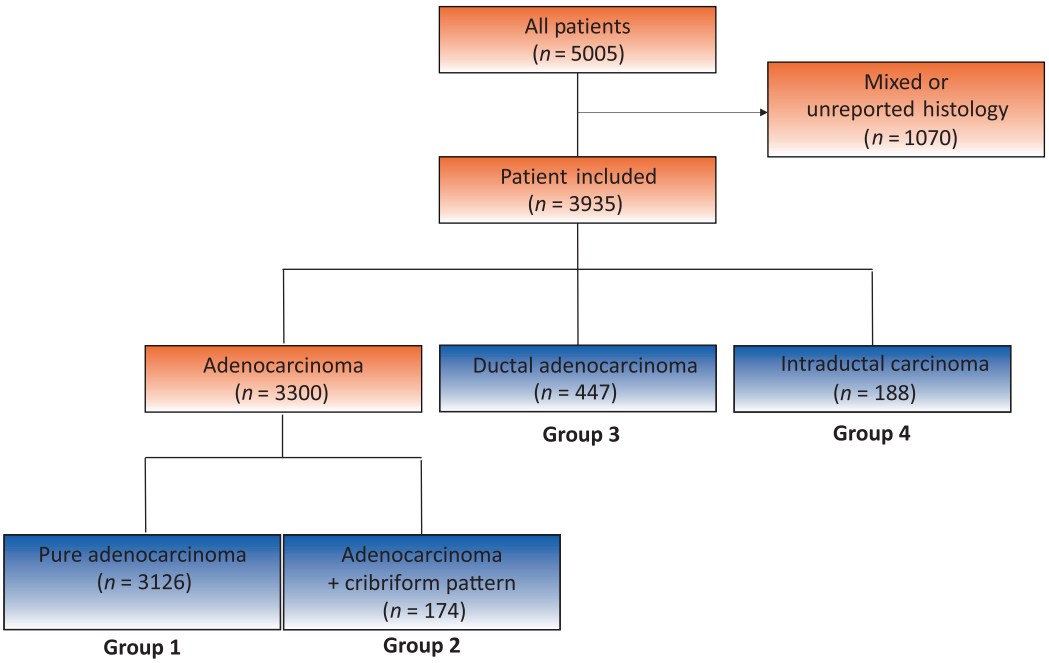
(A)



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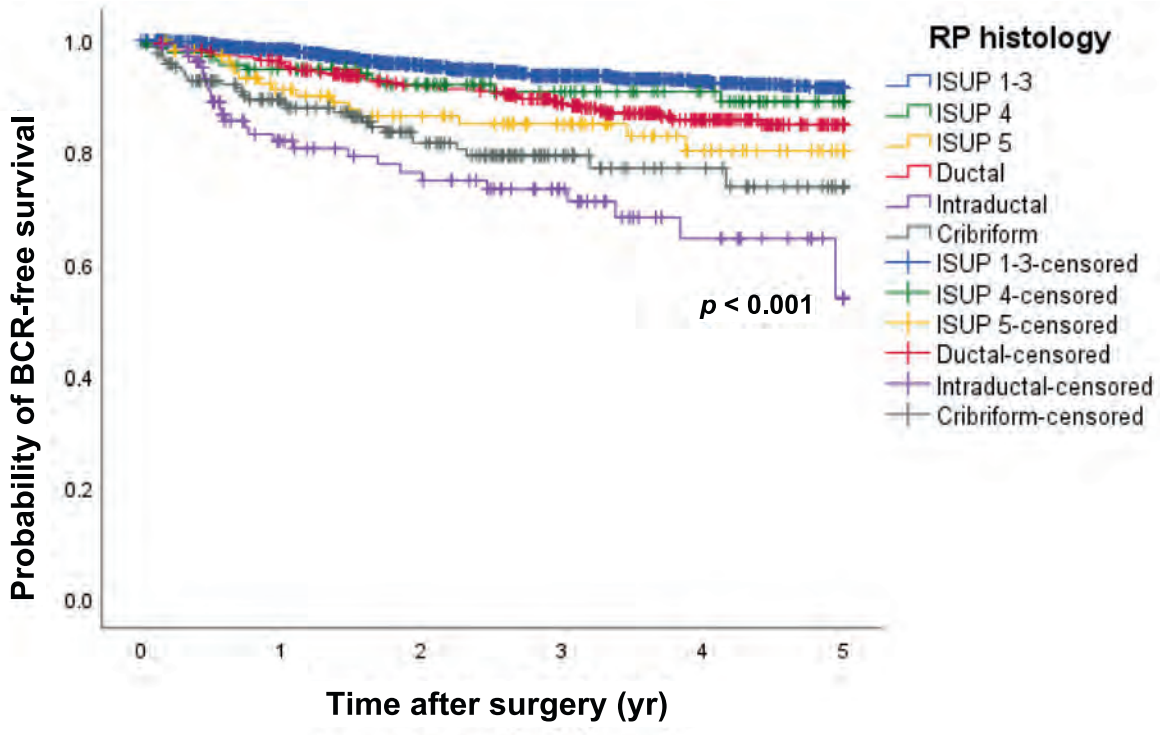

Fig. 1 – (A) Histology groups and the number of patients in each group. Four groups were compared in the analysis: group 1 = pure adenocarcinoma; group 2 = adenocarcinoma with cribriform pattern; group 3 = ductal adenocarcinoma; and group 4 = intraductal carcinoma. (B) Biochemical recurrence (BCR)-free survival after robot-assisted radical prostatectomy by International Society of Urological Pathology (ISUP) grade group and unusual histology groups.

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Table 1 – Baseline patient and disease characteristics

Parameter	Pure AC (*n* = 3126)	DAC (*n* = 447)	*p* value	IDC (*n* = 188)	*p* value	CP (*n* = 174)	*p* value	Overall (*n* = 3935)
Age (yr)[a]	65 (60–70)	65 (60–70)	0.683	67 (61–71)	0.043	67 (62–70)	0.015	65 (60–70)
PSA (ng/ml)[a]	6.9 (5.0–10.0)	6.1 (4.3–8.5)	<0.001	7.2 (5.0–12.1)	0.365	6.5 (5.1–9.0)	0.243	6.8 (5.0–10.0)
Prostate size (cm^3)[a]	39 (28–54)	37 (30–52)	0.733	41 (32–59)	0.054	33 (25–50)	0.001	38 (28–53)
RP pathology, *n* (%)			<0.001		<0.001		<0.001	
GG 1	815 (26.1)	73 (16.3)		1 (0.5)		1 (0.6)		890 (22.6)
GG 2	1123 (35.9)	213 (47.7)		63 (33.5)		98 (56.3)		1497 (38.0)
GG 3	488 (15.6)	85 (19.0)		77 (41.0)		44 (25.3)		694 (17.6)
GG 4	240 (7.7)	21 (4.7)		14 (7.4)		17 (9.8)		292 (7.4)
GG 5	164 (5.2)	21 (4.7)		22 (11.7)		14 (8.0)		221 (5.6)
Data missing	296 (9.5)	34 (7.6)		11 (5.9)		0 (0)		341 (8.7)

AC = adenocarcinoma; CP = cribriform pattern; DAC = ductal AC; GG = International Society of Urological Pathology grade group; IDC = intraductal carcinoma; RP = radical prostatectomy.
[a] Results are reported as the median (interquartile range).

Table 2 – Oncological outcomes

Parameter	Pure AC (*n* = 3126)	DAC (*n* = 447)	*p* value	IDC (*n* = 188)	*p* value	CP (*n* = 174)	*p* value	Total (*N* = 3935)
Surgical margin, *n* (%)			0.039		<0.001		<0.001	
Positive	854 (27.3)	150 (33.6)		80 (42.6)		75 (43.1)		1159 (29.5)
Negative	2009 (64.3)	282 (63.1)		95 (50.5)		96 (55.2)		2482 (63.1)
Data missing	263 (8.4)	15 (3.4)		13 (6.9)		3 (1.7)		294 (7.5)
LNI, *n* (%)			<0.001		<0.001		0.273	
Yes	16 (0.5)	31 (6.9)		15 (8.0)		4 (2.3)		66 (1.7)
No	781 (25.0)	236 (52.8)		51 (27.1)		99 (56.9)		1167 (29.7)
Missing	2329 (74.5)	180 (40.3)		122 (64.9)		71 (40.8)		2702 (68.7)
Adjuvant RT, *n* (%)			0.003		<0.001		0.902	
Yes	141 (4.5)	28 (6.3)		21 (11.2)		9 (5.2)		199 (5.1)
No	2400 (76.8)	251 (56.2)		93 (49.5)		160 (92.0)		2904 (73.8)
Data missing	585 (18.7)	168 (37.6)		74 (39.4)		5 (2.9)		832 (21.1)
Salvage RT, *n* (%)			<0.001		0.002		0.214	
Yes	239 (7.6)	23 (5.1)		16 (8.5)		21 (12.1)		299 (7.6)
No	2200 (70.4)	36 (8.1)		61 (32.4)		143 (82.2)		2440 (62.0)
Data missing	687 (22.0)	388 (86.8)		111 (59.0)		10 (5.7)		1196 (30.4)
BCR, *n* (%)			0.671		<0.001		0.078	
Yes	334 (10.7)	48 (10.7)		32 (17.0)		30 (17.2)		444 (11.3)
No	2203 (70.5)	295 (66.0)		85 (45.2)		137 (78.7)		2720 (69.1)
Data missing	589 (18.8)	104 (23.3)		71 (37.8)		7 (4.0)		771 (19.6)
Nodal recurrence, *n* (%)			<0.001		0.325		0.049	
Yes	11 (0.4)	7 (1.6)		1 (0.5)		5 (2.9)		24 (0.6)
No	1045 (33.4)	24 (5.4)		34 (18.1)		156 (89.7)		1259 (32.0)
Data missing	2070 (66.2)	416 (93.1)		153 (81.4)		13 (7.5)		2652 (67.4)
Metastasis, *n* (%)			0.002		0.002		1	
Yes	29 (0.9)	5 (1.1)		7 (3.7)		4 (2.3)		45 (1.1)
No	1181 (37.8)	28 (6.3)		57 (30.3)		160 (92.0)		1426 (36.2)
Data missing	1916 (61.3)	414 (92.6)		124 (66.0)		10 (5.7)		2464 (62.6)
Status, *n* (%)			0.007		1		1	
Alive	2592 (82.9)	445 (99.6)		139 (73.9)		166 (95.4)		3342 (84.9)
Dead	43 (1.4)	0 (0)		2 (1.1)		2 (1.1)		47 (1.2)
Unknown	491 (15.7)	2 (0.4)		47 (25.0)		6 (3.4)		546 (13.9)
Median follow-up, d (interquartile range)	1039 (553–1674)	1223 (803–1647)	<0.001	888 (357–1212)	<0.001	867 (480–1190)	<0.001	1045 (573–1616)

AC = adenocarcinoma; BCR = biochemical recurrence; CP = cribriform pattern; DAC = ductal AC; IDC = intraductal carcinoma; LNI = lymph node involvement; RP = radical prostatectomy; RT = radiation therapy.

Table 3 – Univariable Cox regression results for unconventional histologies associated with 5-yr biochemical recurrence versus pure adenocarcinoma as the reference

Histology	HR (95% CI)	*p* value
Ductal adenocarcinoma	1.67 (1.16–2.40)	0.005
Intraductal carcinoma	5.22 (3.41–8.01)	<0.001
Cribriform pattern	3.45 (2.29–5.20)	<0.001

CI = confidence interval; HR = hazard ratio.

status were not significant predictors. Conversely, nerve-sparing surgery (HR 0.75; $p = 0.005$) and lymph node dissection (HR 0.58; $p < 0.001$) were associated with lower incidence of erectile dysfunction.

3.4. aRT rates

The use of aRT in all the participating centers was in accordance with the EAU guidelines [5]. aRT use was more fre-

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Table 4 – Multivariable Cox regression results for factors associated with 5-yr biochemical recurrence

Variable	HR (95% CI)	*p* value
RP histology		
AC GG 1–3	Reference	
AC GG 4	2.07 (0.88–4.90)	0.096
AC GG 5	2.02 (0.87–4.68)	0.101
Ductal AC	3.15 (1.72–5.78)	<0.001
Intraductal carcinoma	5.63 (2.74–11.58)	<0.001
Cribriform pattern	3.94 (2.14–7.25)	<0.001
Age	1.00 (0.97–1.03)	0.985
Log PSA	1.63 (1.21–2.19)	0.001
Nerve-sparing surgery	0.63 (0.40–0.99)	0.047
Pathological T stage		
pT2	Reference	
pT3a	1.56 (0.92–2.64)	0.097
pT3b	2.19 (1.24–3.85)	0.007
Positive surgical margin	1.47 (0.97–2.24)	0.069
Positive lymph node	0.88 (0.42–1.83)	0.724

AC = prostate adenocarcinoma; CI = confidence interval; GG = International Society of Urological Pathology grade group; HR = hazard ratio; IDC = intraductal carcinoma; PSA prostate-specific antigen; RP = radical prostatectomy.

Table 5 – Association of unconventional histologies with functional outcomes at 1 yr postoperatively in comparison to pure adenocarcinoma

Histology	Odds ratio (95% confidence interval)	
	Incontinence	Erectile dysfunction
Ductal adenocarcinoma	1.03 (0.77–1.39); $p = 0.839$	1.95 (1.58–2.42); $p < 0.001$
Intraductal carcinoma	1.51 (0.92–2.50); $p = 0.104$	2.63 (1.85–3.73); $p < 0.001$
Cribriform pattern	1.97 (1.33–2.92); $p < 0.001$	3.03 (1.82–5.05); $p < 0.001$

Table 6 – Logistic regression results for the risk of erectile dysfunction at 1 yr

Risk factor	HR (95% CI)	*p* value
RP histology		
AC GG 1–3	Reference	
AC GG 4	0.94 (0.67–1.31)	0.700
AC GG 5	0.76 (0.50–1.16)	0.201
Ductal AC	2.13 (1.67–2.70)	<0.001
Intraductal carcinoma	2.14 (1.45–3.17)	<0.001
Cribriform pattern	2.01 (1.18–3.45)	0.011
Age	1.00 (0.98–1.01)	0.477
Log PSA	1.14 (1.00–1.30)	0.057
Nerve-sparing surgery	0.75 (0.62–0.92)	0.005
Lymph node dissection	0.58 (0.47–0.70)	<0.001
Pathological T stage		
pT2	Reference	
pT3a	1.67 (1.38–2.02)	<0.001
pT3b	1.52 (1.15–2.00)	0.003
pT4	1.69 (0.15–19.02)	0.673
Positive surgical margin	0.99 (0.82–1.19)	0.898

AC = prostate adenocarcinoma; CI = confidence interval; GG = International Society of Urological Pathology grade group; HR = hazard ratio; IDC = intraductal carcinoma; PSA prostate-specific antigen; RP = radical prostatectomy.

quent in DAC (6.3%; $p = 0.003$) and IDC (11.2%; $p < 0.001$) than in AC (4.5%).

Logistic regression analysis with AC GG 1–3 as the reference revealed that IDC was associated with a higher likelihood of aRT receipt (odds ratio [OR] 27.3, 95% confidence interval [CI] 6.79–109.7; $p < 0.001$; Table 7). DAC was also associated with a higher likelihood of aRT receipt (OR 3.27, 95% CI 1.25–8.55; $p = 0.015$). Nodal metastasis (OR 9.09, 95% CI 2.49–33.24; $p < 0.001$), and seminal vesicle invasion (OR 8.13, 95% CI 3.51–18.8; $p < 0.001$) were the other significant predictors. CP (OR 4.2, 95% CI 0.08–2.27; $p = 0.313$), GG 4 and GG 5 disease, positive margin status, and PSA were not significant predictors for aRT receipt.

Table 7 – Logistic regression results for the odds of receipt of adjuvant radiation therapy

Risk factor	Odds ratio (95% CI)	*p* value
RP histology		
AC GG 1–3	Reference	
AC GG 4	3.41 (0.96–12.09)	0.058
AC GG 5	0.70 (0.12–3.94)	0.686
Ductal AC	3.27 (1.25–8.55)	0.015
Intraductal carcinoma	27.31 (6.79–109.74)	<0.001
Cribriform pattern	0.42 (0.08–2.27)	0.313
Log PSA	1.60 (0.91–2.80)	0.101
Positive surgical margin	1.08 (0.48–2.41)	0.856
Seminal vesicle invasion	8.13 (3.51–18.82)	<0.001
Positive lymph node	9.09 (2.49–33.24)	<0.001

AC = prostate adenocarcinoma; CI = confidence interval; GG = International Society of Urological Pathology grade group; PSA = prostate-specific antigen; RP = radical prostatectomy.

Table 8 – Univariable logistic regression results for unconventional histologies associated with receipt of adjuvant radiation therapy versus pure adenocarcinoma as the reference

Histology	HR (95% CI)	*p* value
Ductal adenocarcinoma	1.90 (1.24–2.91)	0.003
Intraductal carcinoma	3.84 (2.32–6.36)	<0.001
Cribriform pattern	0.96 (0.48–1.91)	0.902

CI = confidence interval; HR = hazard ratio.

In addition, aRT was more likely to be required in DAC (HR 1.9) and in IDC (HR 3.84) than in AC according to univariable logistic regression analysis (Table 8).

4. Discussion

In this large multicenter cohort of men with localized PCa treated with RARP, the presence of any UH (DAC, CP, or IDC) was significantly associated with three to five times higher risk of BCR at 5 years in comparison to GG 1–3 pure AC (Table 4). Moreover, logistic regression analyses revealed that the risk of aRT receipt was three times higher for DAC and 27 times higher for IDC in comparison to AC GG 1–3. Regarding functional outcomes, the risk of new-onset erectile dysfunction at one year after RARP was consistently two-fold higher for all UH subtypes in comparison to AC GG 1–3 (Table 6). In addition, CP was associated with a higher risk of urinary incontinence.

To the best of our knowledge, this is the first large multicenter study to evaluate the impact of PCa UH on functional and oncological outcomes after RARP and on the rate of aRT receipt.

UHs are not as rare as conventionally perceived, and an accurate pathological description is mandatory. A systematic review by Porter et al. [15] highlighted that IDC incidence could reach 36.7% in high-risk disease and 56% in

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metastatic or recurrent disease. A review by Montironi et al. [25] showed that IDC was strongly associated with aggressive PCa with high Gleason score and large tumor volume, and usually had a deleterious impact on prognosis. The authors suggested that pathologists should report IDC in prostate specimens, especially from prostate biopsy, because its presence has a critical impact on patient management.

Ericson et al. [26] reported that prostate biopsy had sensitivity of 56.5% and specificity of 87.2% for detection of CP and/or IDC after RP, and that magnetic resonance imaging/ultrasound–guided fusion prostate biopsy did not improve UH detection. Although biopsy cannot confidently rule out UH, patients with biopsy-proven UH histology (especially IDC) should be informed of the higher risk of BCR and aRT receipt, and advised to receive active treatment. In addition, post-RARP diagnosis of UH should trigger discussion about aRT as an option, or at least stricter follow-up in order to pick up the relatively early BCR observed in our study.

Reports on adverse oncological behaviors of these UHs are by no means isolated, and therefore these PCa entities warrant more attention from urologists. Kweldam et al. [10] showed that among the different Gleason 4 grade patterns, CP was independently associated with inferior metastasis-free survival and disease-specific mortality rates after RP among patients with Gleason 7 PCa. In the 2005–2018 cohort described by Ranasinghe et al. [14], DAC treated with either RP or RT was associated with worse 5-yr metastasis-free and overall survival rates in comparison to high-risk PCa. Besides the systematic review by Russo et al. [27] showing inferior oncological outcomes for CP, a recent study found that CP cases typically had a worse response to androgen blockade, suggesting that this UH could contribute to hormonal deprivation resistance [28]. In addition, CP was associated with a higher risk of lymph node positivity at RP [29]. In our analysis, IDC was the strongest predictor of BCR on Cox regression analysis, with a 5.5-fold higher risk. This reiterates the importance for uropathologists to actively look for and report PCa UH.

Several unanswered questions remain for the management of UH PCa. whether there are differences in response to RT (especially for IDC), multimodal therapy, androgen deprivation therapy in the (neo-)adjuvant and palliative settings, novel hormonal agents, or chemotherapy, as well as optimal follow-up protocols. In addition, genomic alterations have been linked to the development of IDC and CP subtypes [30]. Future trials are therefore needed to elucidate the role of genetics and the optimal management for patients with these PCa UH types.

Functional outcomes after RARP are clearly multifactorial and affected by intraoperative (eg, nerve-sparing surgery, urethral length, and strategies for bladder neck preservation and reconstruction) and postoperative factors (eg, rehabilitation programs). Our results can be explained in part by the higher aRT rates for UH. Nevertheless, this study does shed some light on the myths of unconventional PCa histology, and help substantiate our patient counselling. Further studies are warranted to elucidate the association between UH and inferior functional outcomes.

Our study has several limitations: (1) the retrospective nature of the data collected; (2) the lack of central review of histological specimens; (3) the lack of preoperative imaging details and data for patients treated primarily with RT; (4) the omission of rarer UH entities such as mucinous/neuroendocrine disease; (5) the use of nonstandardized follow-up protocols among the participating centers; and (6) the relatively short follow-up (2.8 yr) for our series. The authors hope this cohort acts as a milestone in our pursuit for the optimal management of rarer disease, although further prospective studies are certainly needed to confirm our findings.

5. Conclusions

For patients with PCa treated with RARP, the presence of UH (CP, DAC, or IDC) was associated with a higher rate of early BCR in comparison to pure AC (GG 1–3). Moreover, the presence of DAC or IDC predicted a higher rate of aRT receipt. In terms of functional results, presence of any of the three UH types investigated here predicted two-fold higher risk of erectile dysfunction at 1 yr after RARP, and CP was also associated with higher risk of incontinence. Patients diagnosed with these UHs should be well counselled about such risk, followed up more stringently, and educated about the higher likelihood of multimodal treatment.

Acknowledgement

We would like to acknowledge Dr. Kimberly Chan (Lister Hospital, UK); Dr. Chris Wong, Alex Liu, Ivan Ko, Alex Mok and Mr. Steven Leung (CUHK/ Prince of Wales Hospital, HK); and Dr. Sunny Tsang (Queen Mary Hospital, HK) for their input in data collection and analyses.

Author contributions: Jeremy Yuen-Chun Teoh had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Teoh, Leung, Castellani.
Acquisition of data: All authors.
Analysis and interpretation of data: Leung, Nicoletti.
Drafting of the manuscript: Leung, Nicoletti.
Critical revision of the manuscript for important intellectual content: Castellani, Mazzucchelli.
Statistical analysis: Teoh, Leung, Nicoletti.
Obtaining funding: None.
Administrative, technical, or material support: None.
Supervision: Castellani, Teoh.
Other: None.

Financial disclosures: Jeremy Yuen-Chun Teoh certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

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Funding/Support and role of the sponsor: None.

Data sharing statement: Data are available for bona fide researchers on request from the authors.

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scientific reports

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Tumor localization by Prostate Imaging and Reporting and Data System (PI-RADS) version 2.1 predicts prognosis of prostate cancer after radical prostatectomy

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An improved reading agreement rate has been reported in version 2.1 (v2.1) of the Prostate Imaging and Reporting and Data System (PI-RADS) compared with earlier versions. To determine the predictive efficacy of bi-parametric MRI (bp-MRI) for biochemical recurrence (BCR), our study assessed PI-RADS v2.1 score and tumor location in Japanese prostate cancer patients who underwent radical prostatectomy. Retrospective analysis was performed on the clinical data of 299 patients who underwent radical prostatectomy at Chiba University Hospital between 2006 and 2018. The median prostate-specific antigen (PSA) level before surgery was 7.6 ng/mL. Preoperative PI-RADS v2.1 categories were 1–2, 3, 4, and 5 in 35, 56, 138, and 70 patients, respectively. Tumor location on preoperative MRI was 107 in the transition zone (TZ) and 192 in the peripheral zone (PZ). BCR-free survival was significantly shorter in the PZ group (p = 0.001). In the total prostatectomy specimens, preoperative PI-RADS category 5, radiological tumor location, pathological seminal vesicle invasion, and Grade Group ≥ 3 were independent prognostic factors of BCR. These four risk factors have significant potential to stratify patients and predict prognosis. Radiological tumor location and PI-RADS v2.1 category using bp-MRI may enable prediction of BCR following radical prostatectomy.

Prostate cancer was the second most common male cancer and the fifth leading cause of cancer death worldwide in 2020 (GLOBOCAN 2020)[1]. More than 1.4 million new cases and 375,000 deaths due to prostate cancer are estimated to occur globally per year. Radical prostatectomy remains one of the standard treatments procedure for localized prostate cancer, whereas active surveillance enhances clinical benefits for the low-risk group of prostate cancer[2]. However, pathological Grade Group (GG) may occasionally be overestimated or underestimated in patients who undergo radical prostatectomy for locally advanced prostate cancer at the initial biopsy. Misclassification of tumor risk at diagnosis leads to inadequate treatment, which is associated with inferior outcomes that include BCR and worse survival. The precise staging and estimation of malignancy are essential in the treatment strategies for localized prostate cancer.

In the diagnosis of prostate cancer, detection and localization of malignant lesions are performed using MRI[3]. The Prostate Imaging and Reporting and Data System (PI-RADS) was issued in 2012 by the European Society of Urogenital Radiology (ESUR) as a standardized guideline for the imaging and interpretation of prostate MRI. PI-RADS is also used in evaluating and reporting of prostate cancer on multiparametric MRI (mp-MRI)[4]. In 2015, the ESUR published PI-RADS v2.0[5], followed by the revised PI-RADS v2.1 in 2019[6]. In PI-RADS v2.1, some cases changed the TZ category from 2 to 1 or 3. TZ assessment for category 2 lesions requires background assessments. TZ nodules that were 2 points in PIRADS v2 are downgraded to 1 point if the nodule is similar

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to the background. For a case with T2W score of 2, if the DWI score is 4 or 5, the overall PI-RADS category is upgraded from 2 to 3.

A previous study has indicated the equivalent utility of bi-parametric MRI (bp-MRI) and multi-parametric MRI (mp-MRI)[7]. The clinical value of PI-RADS v2.0 with bp-MRI and pathological Grade Group to predict BCR following radical prostatectomy also has been reported[8]. Patients with renal dysfunction or an allergy to contrast agent are not able to undergo dynamic contrast-enhanced (DCE) MRI. Investigation of a method for detection of prostate cancer in these patients is a pressing clinical issue. In this regard, bp-MRI can be used without contrast agent for imaging prostate tumors. Although PI-RADS v2.1 based on MRI has become the standard option for evaluation of the prostate, as yet there is limited evidence regarding PI-RADS v2.1 and the prediction of BCR after prostatectomy, particularly for bp-MRI. There is also evidence that tumor location influences the prognosis of localized prostate cancer[9]. Based on this evidence, we hypothesize that tumor location as well as MRI findings influence the outcome of radical prostatectomy.

Therefore, the aim of the present study was to examine the prognostic significance of the bp-MRI findings of prostate cancer for BCR, including location and PI-RADS v2.1 category.

Results

Patient characteristics. Table 1 lists the characteristics of the 299 patients that were analyzed in our study. Median follow-up was 49.8 months after radical prostatectomy, median PSA (ng/mL) was 7.6 ng/mL, and median age at operation was 67 years. Open radical prostatectomy (ORP), laparoscopic radical prostatectomy (LRP), and robotic-assisted radical prostatectomy (RARP) were performed in 33 (11.0%), 76 (25.4%), and 190 (63.5%) patients, respectively. Lymph node dissection was performed in 234 patients (78.3%). The PI-RADS v2.1 category of the index tumor was 1–2, 3, 4, and 5 in 35 (11.7%), 56 (18.7%), 138 (46.2%), and 70 (23.4%) patients, respectively. Of the 299 patients, 71 (23.7%) had extra-prostatic extension and 89 (29.8%) specimens had a positive resection margin. Seminal vesicle invasion was found in 28 (9.4%) of patients. Pathological Grade Groups 1, 2, 3, 4, and 5 were diagnosed in 23 (7.7%), 123 (41.1%), 93 (31.1%), 24 (8.0%), and 35 (11.7%) of patients, respectively (Table 1).

Forty-eight patients (16.1%) experienced BCR during the observation period. Baseline PSA, PI-RADS category, radiological location, Pathological Grade Group, resection margin positive (RM+), and seminal vesicle invasion positive (SV+) results were significantly different between the two groups of patients with or without biochemical failure (Table 2).

Cox proportional hazard models for BCR. Univariate Cox proportional hazard model identified the following as significant factors for BCR: initial PSA ≥ 7.6 ng/mL ($p = 0.0319$), extra-prostatic extension (EPE) positive ($p < 0.0001$), RM+ ($p < 0.0001$), SV+ ($p < 0.0001$), Pathological Grade Group ≥ 3 ($p < 0.0001$), lymph node metastases ($p = 0.013$), radiological tumor location at PZ ($p = 0.002$), and PI-RADS category 5 ($p < 0.0001$) (Table 3).

Characteristics	
Total patients	299
Median age at surgery (range), y	67 (46–77)
Median PSA (range), ng/mL	7.6 (2.3–87.16)
PI-RADS v2.0 score, n (%)	
1–2/3/4/5	66 (22.1%)/25 (8.4%)/138 (46.2%)/70 (23.4%)
PI-RADS v2.1 score, n (%)	
1–2/3/4/5	35 (11.7%)/56 (18.7%)/138 (46.2%)/70 (23.4%)
Radiological location (TZ/PZ)	107/192
Surgical approach n (%)	
Open/laparoscopic/robot-assisted	33 (11.0%)/76 (25.4%)/190 (63.5%)
Lymph node dissection, n (%)	234 (78.3%)
Pathological Grade Group, n (%)	
1/2/3/4/5	23 (7.7%)/123 (41.1%)/93 (31.1%)/24 (8.0%)/35 (11.7%)
Undiagnosed	1 (0.3%)
Extraprostatic extension (EPE1), n (%)	71 (23.7%)
Resection margin (RM+), n (%)	89 (29.8%)
Seminal vesicle invasion (SV+), n (%)	28 (9.4%)
Lymph node metastasis (N1), n (%)	4 (1.3%)
Median observation period (months)	49.8
Biochemical failure, n (%)	48 (16.1%)

Table 1. Patient characteristics. *PSA* prostate-specific antigen, *PI-RADS* Prostate Reporting and Imaging and Data System, *TZ* transition zone, *PZ* peripheral zone.

Characteristic	With BCR	Without BCR	*p* value
No. patients (%)	48 (16.1%)	251 (83.9%)	–
Median baseline PSA (range), ng/mL	10.61 (4.15–47.35)	7.22 (2.3–87.16)	0.0026**
PI-RADS v2.1 category, n	1 (0), 2 (1), 3 (7), 4 (15), 5 (25)	1 (19), 2 (15), 3 (49), 4 (123), 5 (45)	< 0.0001**
PI-RADS v2.1 category 5, n (%)	25 (52.1%)	45 (17.9%)	< 0.0001**
Radiological location, TZ/PZ	8 (16.7%)/40 (83.3%)	99 (39.4%)/152 (60.6%)	0.0075**
Pathological Grade Group 3–5, n	40 (83.3%)	112 (44.6%)	< 0.0001**
Resection margin positive, n (%)	30 (62.5%)	59 (23.5%)	< 0.0001**
Seminal vesicle invasion, n (%)	15 (31.3%)	13 (5.18%)	< 0.0001**
Lymph node metastasis, n (%)	2 (4.2%)	2 (0.8%)	0.1763

Table 2. Clinical characteristics according to presence or absence of BCR. *PSA* prostate-specific antigen, *PI-RADS* Prostate Reporting and Imaging and Data System, *BCR* biochemical recurrence, *TZ* transition zone, *PZ* peripheral zone. $^{**}p < 0.01$.

	Univariate			Multivariate		
Variable	HR	95%CI	*p* value	HR	95%CI	*p* value
Age at surgery > 67 y	1.02	0.57–1.81	0.9489			
Initial PSA > 7.63	1.92	1.06–3.48	**0.0319***	1.24	0.65–2.34	0.5169
EPE positive	5.39	2.88–10.08	**< 0.0001****	1.31	0.57–2.99	0.7719
RM positive	4.59	2.49–8.46	**< 0.0001****	2.17	1.01–4.68	0.083
SV invasion positive	8.54	4.54–16.06	**< 0.0001****	2.65	1.26–5.58	**0.0103***
Grade Group 3–5	6.40	2.86–14.31	**< 0.0001****	2.82	1.20–6.64	**0.0174***
Lymph node metastases	6.07	1.46–25.18	**0.013***	2.31	0.49–10.9	0.2927
tumor location (PZ)	3.56	1.59–7.97	**0.002****	2.96	1.23–7.11	**0.0157***
PI-RADS category 5	4.20	2.36–7.44	**< 0.0001****	2.8	1.48–5.29	**0.0015****

Table 3. Uni- and multivariate Cox proportional hazard models for BCR-free survival. Significant values are in bold. *PSA* prostate-specific antigen, *EPE* extraprostatic extension, *RM* resection margin, *SV* seminal vesicle, *PZ* peripheral zone, *PI-RADS* Prostate Reporting and Imaging and Data System, *BCR* biochemical recurrence, *HR* hazard ratio, *CI* confidence interval. $^{*}p < 0.05$; $^{**}p < 0.01$.

Multivariate analysis identified the following as independent risk factors for BCR: Pathological Grade Group ≥ 3 (p = 0.0174), radiological tumor location at PZ (p = 0.0157), seminal vesicle invasion positive (p = 0.0103), and PI-RADS category 5 (p = 0.0015).

Kaplan–Meier analysis. We performed Kaplan–Meier analysis to analyze factors identified as significant in the multivariate Cox proportional hazard model, which were radiological location at PZ (p = 0.001) (Fig. 1A), pathological Grade Group 3 (Fig. 1B), seminal vesicle invasion (SV+) (Fig. 1C), and PI-RADS category 5 (Fig. 1D) (all p < 0.0001). We built our original prediction model based on these four risk factors accordingly.

Prognostic model for BCR using v2.1. We propose a new scoring system that classifies the risk categories by the four factors (Pathological Grade Group ≥ 3, radiological location at PZ, seminal vesicle invasion, and PI-RADS category 5) predictive of BCR after radical prostatectomy (Fig. 2A). One point is assigned for each positive factor, and the points are summed to give the total score. We divided the patients into three groups according to the summed score, as follows: score 0–2, low-risk group; 3 points, intermediate-risk group; and 4 points, high-risk group. There were 248 (82.9%), 39 (13.0%), and 12 (4.0%) patients in the low-, intermediate-, and high-risk groups, respectively. The Kaplan–Meier method was used to evaluate prognosis. Prognosis for BCR was the worst in the high-risk group. This novel prognostic model for BCR, which takes into account PI-RADS v2.1 as well as clinical factors, enables differentiation of patients according to risk factors for PFS between high- and intermediate-risk (p = 0.0065), intermediate- and low-risk (p < 0.0001), and low- and high-risk groups (p < 0.0001) (Fig. 2B).

Radiological location as a preoperative predictive factor. Radiological location in the PZ was a worse prognostic factor than in the TZ (Fig. 1A). Patients with tumors in the radiological TZ had a lower BCR rate (7.5%) compared with those in the radiological PZ (20.8%) (p = 0.0075) (Table 2). We divided patients into two groups according to the radiological location (radiological TZ and PZ groups).

The univariate Cox proportional hazard model found no factors of significance for BCR in the TZ group, whereas the PZ group showed significant differences in terms of EPE positive (p < 0.0001), RM positive (p < 0.0001), SV positive (p < 0.0001), GG ≥ 3 (p = 0.0003), lymph node metastases (p = 0.0388), and PI-RADS

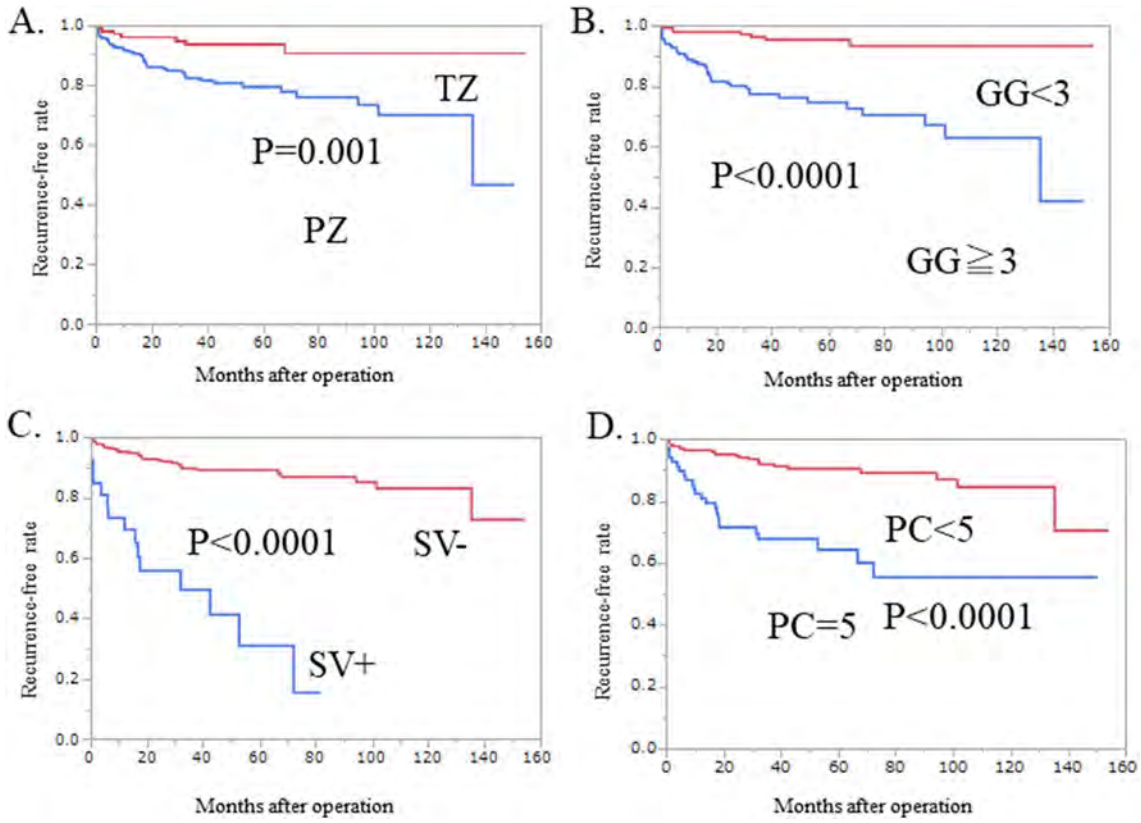

Figure 1. Kaplan–Meier analysis of factors identified as significant for BCR in the multivariate Cox proportional hazard model. (**A**) Radiological location. PFS in BCR was worse in tumors with radiological location in the PZ than in the TZ ($p = 0.001$). (**B**) Pathological Grade Group (GG). (**C**) Seminal vesicle invasion. (**D**) PI-RADS category (PC) 5. Tumors with Grade Group (3–5), seminal vesicle invasion (SV+), and PI-RADS category 5 had worse PFS in BCR ($p < 0.0001$, $p < 0.0001$, and $p < 0.0001$, respectively) compared with Grade Group (1–2), SV-, and PI-RADS category (1–4).

category 5 ($p < 0.0001$). Furthermore, multivariate analysis identified RM positive ($p = 0.0219$), SV positive ($p = 0.0114$), Grade Group ≥ 3 ($p = 0.0201$), and PI-RADS category 5 ($p = 0.0001$) as independent risk factors (Table 4).

It appears that preoperative PI-RADS location can predict the incidence of postoperative BCR. Patients with tumor in the radiological PZ region are more likely to suffer BCR if this finding is combined with the above four factors (RM positive, SV positive, Grade group ≥ 3, and PI-RADS category 5) following radical prostatectomy.

Effect of radiological localization on efficacy of predictive factors. Tumors located in the TZ had a better prognosis for BCR (Table 4). Kaplan–Meier analysis among the radiological PZ tumors identified PI-RADS category 5 ($p < 0.0001$) and Grade Group ≥ 3 ($p < 0.0001$) as significant factors predictive of BCR. For tumors located in the TZ, neither of these factors was predictive of BCR ($p = 0.6702$ and $p = 0.2890$, respectively) (Fig. 3).

These results indicate that Grade group ≥ 3 and PI-RADS category 5 could be used to assess the likely occurrence of BCR in PZ tumors, and show that the efficacy of the predictive factors varies according to the radiological location.

Discussion

The present study is the first to report that BCR after radical prostatectomy can be predicted by preoperative MRI tumor location evaluated by PI-RADS v2.1. Our results showed that zonal location of the tumor on preoperative MRI was a significant predictor of BCR. Based on the factors remaining by multivariate analysis for prediction of BCR, we propose a novel risk-classification model based on the following: PZ lesion on MRI, Pathological Grade Group ≥ 3, seminal vesicle invasion, and PI-RADS category 5. Classification of patients into the low-risk (0–2 points), intermediate-risk (3 points), and high-risk (4 points) groups predicted the prognosis of localized prostate cancer patients with statistically significant accuracy. The proposed risk classification system may contribute to the development of treatment strategies for localized prostate cancer.

Takahashi et al. reported that in radical prostatectomy specimens of Japanese patients, approximately 40% of prostate cancer originated in the TZ[10]. Compared to Caucasian men, Japanese patients had a greater incidence of TZ cancer. The pathological characteristics of TZ and PZ cancer are similar except for pathological T stage in

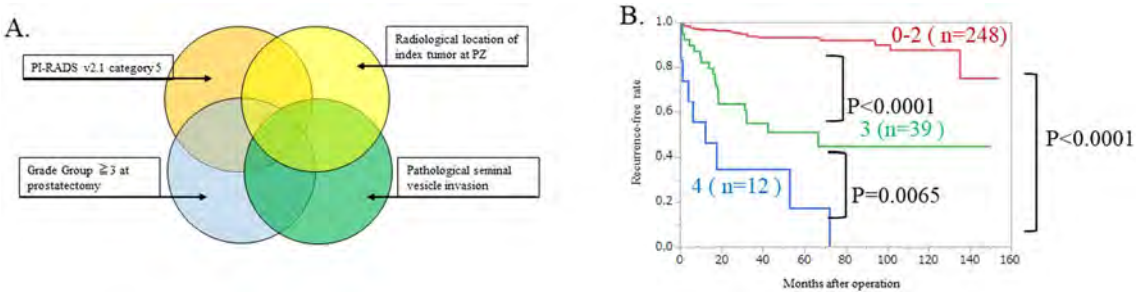

Figure 2. Novel prognostic model for BCR that combines PI-RADS v2.1 and clinical factors. (**A**) Novel prognostic model for BCR. The scoring system classifies the risk category according to the four factors predictive of BCR after radical prostatectomy. (**B**) Kaplan–Meier curve according to the novel prognostic model. The total score is the summed score of all positive factors (one point each). We divided the patients into three groups according to the summed score. Patients with a score of 0–2 were defined as the low-risk group (n = 248), those with 3 points as the intermediate-risk group (n = 39), and those with 4 points as the high-risk group (n = 12). Risk classification significantly differentiated the PFS of BCR between the high- and intermediate-risk, between the intermediate- and low-risk, and between the low- and high-risk groups (p = 0.0065, p < 0.0001, p < 0.0001).

	PZ						TZ		
	Univariate			Multivariate			Univariate		
Variable	HR	95% CI	*p* value	HR	95% CI	*P* value	HR	95% CI	*p* value
Age at surgery > 67 y	1.01	0.54–1.89	0.9653				1.03	0.23–4.65	0.9628
Initial PSA > 7.63	1.64	0.86–3.11	0.1307				3.00	0.58–15.5	0.1895
EPE positive	5.49	2.74–11.03	**<0.0001****	1.38	0.57–3.32	0.478	2.46	0.45–13.4	0.4922
RM positive	4.97	2.46–10.04	**<0.0001****	2.63	1.15–6.03	**0.0219***	1.52	0.29–7.83	0.8832
SV invasion positive	7.78	3.96–15.3	**<0.0001****	2.72	1.25–5.90	**0.0114***	4.83	0.57–41.2	0.1501
Grade Group 3–5	6.75	2.40–19.0	**0.0003****	3.61	1.22–10.6	**0.0201***	2.32	0.47–11.5	0.3032
N+	4.52	1.08–18.9	**0.0388***	2.57	0.54–12.3	0.2381	–	–	–
PI-RADS category 5	5.63	3.00–10.6	**<0.0001****	3.85	1.93–7.70	**0.0001****	0.63	0.76–5.27	0.6729

Table 4. Difference in the predictive factors between the radiological location. Significant values are in bold. *PSA* prostate-specific antigen, *EPE* extraprostatic extension, *RM* resection margin, *SV* seminal vesicle, *N+*: ymph-node positive, *PI-RADS* Prostate Reporting and Imaging and Data System, *PZ* peripheral zone, *TZ* transition zone. *$p < 0.05$; **$p < 0.01$.

the case of autopsy and cystoprostatectomy for bladder cancer[11]. TZ cancers are associated with decreased odds of adverse pathological findings and demonstrate improved recurrence-free survival. These favorable outcomes appear to be the result of different tumor biology[12]. Understanding the biology of tumors originating in different prostate zones will enable zone-specific therapies[13]. The present study revealed that for prediction of BCR, the efficacy of Grade group ≥ 3 and PI-RADS category 5 differed between the radiological TZ and PZ. This risk criterion may predict BCR after radical prostatectomy and enable optimization of zone-specific therapeutic strategy. As discussed in a previous report[13], zone-specific strategies may be considered when choosing between active surveillance, radical prostatectomy, and extended lymph node dissection in patients with Gleason Score and T stage in the same category but in different location. The rationale for the zone-specific strategy may be explained by the difference in the genetic background and biomarker between TZ and PZ, which will lead to the difference in the therapeutic response and prognosis[13].

Previous studies have shown that seminal vesicle invasion and extraprostatic extension predict BCR after radical prostatectomy are related to predictive factors[14–16]. A positive surgical margin affects the incidence of BCR[17,18]. BCR risk is significantly higher for posterior-positive surgical margin than for other positive surgical margins[19]. Broad and anterior positive surgical margin has the highest risk of recurrence after radical perineal prostatectomy[20]. Prognosis was worse in the case of positive seminal vesicle invasion on preoperative MRI compared with negative seminal vesicle invasion[21].

Several reports have evaluated oncological outcomes in patients with negative mp-MRI. Vinayak reported that patients with negative MRI findings (PI-RADS v2.0 score ≤ 2) who underwent radical prostatectomy had oncological outcomes comparable with positive MRI findings (PI-RADS v2.0 score ≥ 3) in terms of clinically significant prostate cancer rates, positive surgical margins, and BCR rates[22]. Shin et al. assessed patients with PI-RADS categories 4–5 on preoperative MRI who underwent prostatectomy and concluded that prognosis was predicted by the location of the lesion on preoperative MRI[23].

In the present study, we analyzed patients with PI-RADS categories 1–5, not just categories 4–5. We found that prognosis was predicted by tumor location in PI-RADS v2.1 category 5 by MRI. To the best of our knowledge,

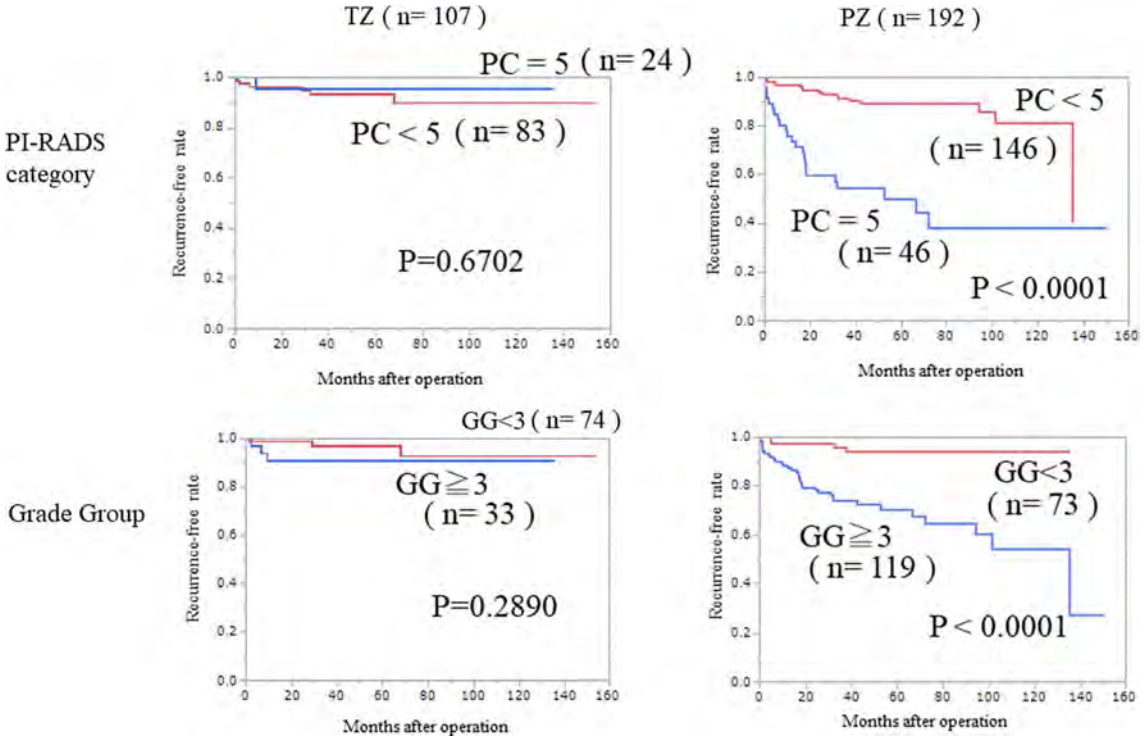

Figure 3. Kaplan–Meier analysis of efficacy of predictive factors according to radiological location. In PZ tumors, PI-RADS category 5 and Grade group ≥ 3 were significant predictive factors of BCR ($p < 0.0001$ and $p < 0.0001$, respectively). In TZ tumors, PI-RADS category 5 and Grade group ≥ 3 were not predictive of BCR ($p = 0.6702$ and $p = 0.2890$, respectively). These findings illustrate that the efficacy of GG ≥ 3 and PI-RADS category 5 differ according to the radiological location of the tumor.

this is the first study to report the ability of zonal location on preoperative MRI to predict post-operative BCR of prostate cancer using PI-RADS v2.1.

Differences in evaluation between PI-RADS v2.0 and PI-RADS v2.1. There are three significant differences between PI-RADS v2.1 and v2.0 in evaluating scoring. First, the definitions of scores 1 and 2 have been revised for TZ lesions on T2WI. Second, on evaluating the total score in TZ, a DWI score of 4 or 5 elevates the overall PI-RADS assessment category from 2 to 3 for lesions receiving a T2WI score of 2. Third, the definitions for DWI scores of 2 and 3 have been revised for lesions located in TZ/PZ. As PI-RADS v2.1 improves inter-reader reproducibility, these revisions may contribute to increased diagnostic performance[6,24]. We have previously reported that bp-MRI and Grade Group predict BCR after radical prostatectomy[8]. In the present study, we analyzed the predictive ability of location on preoperative MRI and evaluation using the new categorization in PI-RADS v2.1 in a large number of patients who underwent radical prostatectomy. In our study, changing to the PI-RADS v2.1 criteria resulted in a change in classification for 40 of the 299 patients. The data of these 40 patients are summarized in Supplementary Table 1.

Limitations. There are several limitations of this study. First, the number of patients analyzed was relatively limited and the evaluations were performed retrospectively. We plan to confirm our results in multi-institutional and prospective settings. Second, the median follow-up period was 49.8 months, and thus assessment related to survival was inadequate. It is necessary to assess oncological outcomes in a longer term. Third, surgery was performed mainly by three surgeons. The differences in prognosis may have been affected by the surgeons' skills. Finally, patients of a single Asian race were investigated in our study. The incidence of and deaths due to prostate cancer are lower in the Asian population than in the Western population[25], which might have some impact on the generalizability of our results.

Conclusion

To the best of our knowledge, this is the first report to evaluate the risk of BCR by radiological tumor location by PI-RADS v2.1 category on preoperative MRI and by pathological diagnosis. We propose a novel risk-classification model based on the following independent risk factors: PZ location on MRI, Pathological Grade Group ≥ 3, seminal vesicle invasion, and PI-RADS category 5. This risk model could be applied to constructing and optimizing treatment strategies for patients with localized prostate cancer.

Materials and methods

Clinical data from 299 patients who had undergone radical prostatectomy at Chiba University Hospital between 2006 to 2018 were retrospectively investigated. Ethics declaration: The study was approved by the Research ethics committee of the graduate school of medicine, Chiba University (approval number 2718). Informed consent was obtained from all participants and/or their legal guardians. The present study was conducted in accordance with ethical standards that promote and ensure respect and integrity for all human subjects and the Declaration of Helsinki. All experiments were performed in accordance with relevant named guidelines and regulations. The clinical factors of Gleason score, pathological features, and clinical tumor location were obtained from the patients' medical records. Radical prostatectomy was performed by one of three surgical approaches (open, laparoscopic, and robot-assisted). Lymph node dissection was performed in 234/299 patients (78.3%). All patients underwent preoperative MRI followed by prostate biopsy and total prostatectomy.

We compared each patient's scores for Prostate Imaging Reporting and Data System (PI-RADS) version 2.1 and version 2.0, based on bp-MRI. Overall survival and BCR-free survival were evaluated by the Kaplan–Meier method.

Definition of PSA progression. Using the definition of the Prostate Cancer Clinical Trial Working Group 2 (PCWG2)[26], we defined BCR as an elevation in PSA of ≥ 0.2 ng/mL after radical prostatectomy, which was confirmed in two consecutive measurements obtained at least 2 weeks apart. We defined the operation date as the date of PSA failure if PSA was ≥ 0.2 ng/mL after radical prostatectomy.

MRI protocol. All enrolled patients underwent prostate MRI at 3 T prior to prostate biopsy. MRI was obtained with T1-weighted, T2-weighted, and diffusion-weighted imaging (DWI), and apparent diffusion coefficient maps were generated with b values of 0 and 1000 s/mm^2. We used a high b-value (b = 2000) for DWI. bp-MRI comprised T2-weighted imaging and DWI. The radiologist used both bp-MRI and the apparent diffusion coefficient maps to determine the PI-RADS score.

PI-RADS v2.1. The PI-RADS scores were evaluated on non-contrast-enhanced bp-MRI by one radiologist (T.H.) with over 10 years of experience in diagnostic radiology. Using the scoring method of PI-RADS v2.1, each patient's score was recorded using a 5-point scale (1–5) and the zonal location. PI-RADS v2.1 was designed to improve detection, location, characterization, and risk stratification in patients with suspected cancer in treatment-naive prostate glands, with the overall objective of improving outcomes for patients. The changes incorporated in PI-RADS v2.1 were revised scoring of DWI in all zones in categories 2–3, and scoring of the overall assessment category in TZ. In TZ, a DWI score of 4 or 5 elevates the overall PI-RADS assessment category from 2 to 3 for lesions that receive a T2W score of 2. PI-RADS v2.1 states that T2-weighted images should be evaluated in the axial plane and in at least one additional orthogonal plane[27].

Statistical analysis. We performed univariate and multivariate Cox proportional hazard analyses to evaluate hazard ratios for BCR-free survival. Cut-offs of continuous variables were selected according to median values. Hazard ratios and 95% confidence intervals were derived. Kaplan–Meier methods were used for survival analysis. Statistical analysis was performed using JMP 14.2.0 (SAS Institute, Cary, NC, USA). Significance was considered at $p < 0.05$.

Data availability

The data sets used and analyzed in the current study are available from the corresponding authors upon reasonable request.

Received: 27 January 2023; Accepted: 8 June 2023
Published online: 21 June 2023

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Acknowledgements

The present study was supported by grants from Grant-in-Aid for Scientific Research (C) (20K09555 to S.S.), Grant-in-Aid for Scientific Research (B) (20H03813 to T.I.), and the Japan China Sasakawa Medical Fellowship (to X.Z.).

Author contributions

A.F. contributed to collecting data, preparing figures, drawing tables, and writing; S.S. contributed to analyzing data, collecting bibliography, and writing; T.H. and Y.Y. contributed to analyzing data. X.Z., J.R., N.T., Y.I., T.S., K.M., and J.I. contributed to collecting data. S.S. and T.I. contributed to the supervision of all activities. The first draft of the manuscript was prepared by A.F., T.H.; and X.Z. performed subsequent amendments. S.S. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-023-36685-1.

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ANTICANCER RESEARCH *43*: 5705-5712 (2023)
doi:10.21873/anticanres.16776

Preoperative PI-RADS v2.1 Scoring System Improves Risk Classification in Patients Undergoing Radical Prostatectomy

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Abstract. *Background/Aim: The purpose of this study was to examine the prognostic value of Prostate imaging-reporting and data system (PI-RADS) v2.1 scoring system in patients who underwent radical prostatectomy (RP). Patients and Methods: Clinical data of 294 patients who received RP between 2006 and 2018 were reviewed and multiple parameters including PI-RADS v2.1 score were employed to identify predictive factors for biochemical recurrence (BCR). Tumor volume was calculated from prostatectomy specimens. Results: Median age at operation and initial PSA level were 67 years old and 7.68 ng/ml, respectively. 44.9 and 24.8% of patients were diagnosed with PI-RADS score 4 and 5 prior to biopsies, respectively. BCR was observed in 17% of patients and median observation period was 63.43 months. After multivariate analysis, PI-RADS v2.1 score 5 [hazard ratio (HR)=2.24, p=0.0124] was an independent predictive factor of BCR in addition to clinical T stage (≥2c) (HR=2.32, p=0.0093) and biopsy Gleason score (≥8) (HR=2.81, p=0.0007). Furthermore, PI-RADS score 5 significantly stratified the prognosis in D'Amico intermediate- and high-risk groups (p=0.0174 and p=0.0013, respectively). We established novel risk classifications including PI-RADS v2.1 score and found that prognostic capabilities were improved as compared to the D'Amico classification. Conclusion: The PI-RADS v2.1 score exhibited significant prognostic value in patients with localized prostate cancer following RP. Risk classifications based on PI-RADS v2.1 score might provide better ability for predicting oncological outcomes as compared to the D'Amico classification system.*

Prostate cancer is the most commonly diagnosed cancer among men with approximately 280,000 cases and the second leading cause of cancer death with 34,700 cases in men in the United States (1, 2). Clinically localized PCa has an extremely favorable prognosis, however, early biochemical recurrence (BCR) may occur in some cases after definitive treatment, requiring adjuvant therapy. Previous studies have attempted to develop useful models to predict prognosis after radical prostatectomy (RP). For instance, the D'Amico classification system was proposed as an optimal staging system following local treatment such as RP and radiotherapy (3). Thereafter, this classification including Gleason score sum (GS), prostate-specific antigen (PSA) level, and clinical T stage, has been widely used for risk stratification in patients with localized PCa. However, better risk-based classification systems using preoperative factors are required to enable better informed decision-making regarding treatment.

The Prostate Imaging-Reporting and Data System (PI-RADS) scoring system was first proposed to represent cancer lesion and aggression using muti-parametric magnetic resonance imaging (mp-MRI) in 2012 by the European Society of Urogenital Radiology (ESUR) (4). Thereafter, several improvements have been made, and version 2.1 is currently in clinical use (5, 6). This system assesses the detectable lesions by mp-MRI and scores the degree of clinically significant cancer lesions (4). The PI-RADS v2.1 scoring system has previously been reported to detect clinically significant prostate cancer, improve the diagnostic accuracy, and avoid unnecessary prostate biopsies (7). However, little is known about its clinical significance as a prognostic predictor after definitive treatment. Given that the PI-RADS scoring system is relevant to likelihood of prostate cancer, in other words, might be related to the size of the cancer lesions on MRI findings, it may be

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Key Words: Prostate cancer, PI-RADS v2.1, radical prostatectomy, risk classification, tumor volume.

useful to develop a risk classification prior to local therapy for predicting oncological outcomes. The D'Amico classification system was originally developed in 1998 and has since been widely applied in approaches for assessing prostate cancer risk (8-10). This classification system was designed to evaluate the risk of recurrence following local treatment of prostate cancer and is used to make more informed decisions regarding their treatment options.

In the present study, we hypothesized that preoperative PI-RADS v2.1 scoring system would improve the accuracy of predicting postoperative BCR by modifying the existing risk classifications such as the D'Amico classification system. Herein, we explored the clinical utility of preoperative PI-RADS v2.1 score and established a novel risk classification for better prediction of clinical outcomes in patients undergoing RP.

Patients and Methods

Patients. Clinical data from 294 patients who underwent RP at Chiba University Hospital and affiliated institutions between 2006 and 2018 were reviewed. Enrolled patients had undergone prostate needle biopsies and diagnosed with prostate adenocarcinoma with GS classification by the pathologists. All patients underwent RP without neoadjuvant hormone therapy using open, laparoscopic, and robot-assisted procedures. The present study was approved by all institutional review boards and informed consent was obtained from all patients.

Data collection. We investigated the following clinical data for each patient: age at operation, initial PSA (iPSA) level prior to biopsies, prostate volume, biopsy GS sum (bGS), clinical TNM classification, and pathological outcomes of the prostate specimen. The following method was used to measure tumor volumes of prostatectomy specimens (11). All specimens were sectioned transversely at 5-mm intervals and submitted as whole sections. If multiple tumors were present, only the index tumor was measured. All slides containing cancer lesions were imported into ImageJ (National Institutes of Health, Bethesda, MD, USA). Tumor volume was determined by scanning the specimen sections and analyzing the area of the tumor using ImageJ. The following formula was used: total tumor volume (ml)=tumor area × specimen thickness × 1.1 (shrinkage corrected) (11).

MRI protocol and PI-RADS v2.1 scoring system. All patients underwent MRI of the prostate at 3T prior to prostate biopsy. MRI was performed using T1-weighted, T2-weighted, and diffusion-weighted imaging (DWI) sequences to produce an apparent diffusion coefficient map. A high b value (b=2,000) was used for DWI. MRI consisted of T2-weighted images and DWI. Both the bi-parametric MRI (bp-MRI) comprising T2-weighted imaging and DWI, and the apparent diffusion coefficient map were employed by the radiologist to determine the PI-RADS v2.1 score.

PI-RADS v2.1 scores were assessed by the radiologist with non-contrast bp-MRI. The score for each patient was documented using the PI-RADS v2.1 scoring method (5-point scale). The modifications implemented in PI-RADS v2.1 were the scoring of DWI in all zones in categories 2-3 and the revised scoring of the overall rating category in the transition zones (TZs). A DWI score of 4 or 5 elevated the overall PI-RADS rating category from 2 to 3 for lesions with a T2W score of 2 in a TZ (12).

Table I. *Patient background*.

No. of patients	294
Median age at diagnosis (range), years	67 (46-77)
Median initial PSA (range), ng/ml	7.68 (0.02-87.16)
Prostate volume (range) ml	29 (10.97-112)
PI-RADS v2.1 score n (%)	
≤3	89 (30.3)
4	132 (44.9)
5	73 (24.8)
Clinical T stage, n (%)	
≤2b	213 (72.9)
≥2c	79 (27.0)
Biopsy GS, n (%)	
≤7	227 (77.2)
≥8	67 (22.8)
Surgical procedures (Open/LRP/RARP), n (%)	33 (11.2)/77 (26.2)/184 (62.6)
Pathological T stage, n (%)	
≤2b	213 (72.9)
≥2c	79 (27.0)
Pathological GS, n (%)	
≤7	231 (79.3)
≥8	60 (20.7)
RM, n (%)	88 (31.2)
Tumor volume (ml)	1.88 (0.02-25.02)
Biochemical recurrence, n (%)	50 (17)
Median observation period (range), months	63.43 (4.87-161.47)

PSA: Prostate-specific antigen; PI-RADS: prostate imaging-reporting and data system; GS: Gleason score; RM: resection margin; LRP: laparoscopic radical prostatectomy; RARP: robot-assisted radical prostatectomy.

Definition of biochemical recurrence (BCR). The Prostate Cancer Clinical Trial Working Group 2 (PCWG2) definition was employed to determine BCR in the present study (13). BCR was defined as a PSA concentration ≥0.2 ng/ml following RP, measured on two consecutive occasions at least 2 weeks apart. The date of surgery was defined as the date of BCR if PSA level was ≥0.2 ng/ml even postoperatively.

Statistical analysis. Student's *t*-test and the χ^2 test were used for comparisons between groups. Kaplan–Meier methods (log-rank test) and Cox proportional hazard models were implemented to evaluate the clinical outcomes and predictive factors. Multivariate analysis was performed with clinical parameters showing statistical significance in univariate analyses. JMP Pro 15 (SAS Institute, Tokyo, Japan) was used to for statistical analyses. Statistical significance was set at the level of $p<0.05$.

Results

Patients background. The study included 294 patients with localized prostate cancer. Median age and PSA level at operation were 67 years old and 7.68 ng/ml, respectively (Table I). Pre-biopsy PI-RADS v2.1 score was 44.9% for score 4 and 24.8% for score 5. Sixty-seven patients (22.8%)

Table II. *Characteristics of patients classified according to the prostate imaging-reporting and data system (PI-RADS) v2.1 score.*

	PI-RADS score		*p*-Value
	≤3 (n=89)	≥4 (n=205)	
Median age (range), years	66 (50-76)	68 (46-77)	0.0465#
Median initial PSA (range), ng/ml	6.35 (0.02-25.31)	8.33 (2.45-87.16)	0.0006#
Median prostate volume (range), ml	30.5 (15.95-112)	28.1 (10.97-106)	0.0004#
Clinical T stage, n (%)			0.0002*
≤2b	81 (94.2)	155 (77.1)	
≥2c	5 (5.8)	46 (22.8)	
Biopsy GS, n (%)			0.0006*
≤7	78 (87.6)	149 (72.7)	
≥8	11 (12.4)	56 (27.3)	
D'Amico classification, n (%)			0.0033*
Low	20 (22.5)	15 (7.3)	
Intermediate	55 (61.8)	125 (61)	
High	14 (15.7)	65 (31.7)	
Pathological T stage, n (%)			<0.0001*
≤2b	42 (48.8)	45 (22.6)	
≥2c	44 (51.2)	154 (77.4)	
Pathological GS, n (%)			0.0228*
≤7	76 (87.4)	154 (75.5)	
≥8	11 (12.6)	50 (24.5)	
RM, n (%)	11 (12.6)	77 (39.5)	<0.0001*
Tumor volume (ml)	0.715 (0.02-8.55)	2.1655 (0.0913-25.02)	<0.0001#
Biochemical recurrence, n (%)	9 (10.1)	41 (20)	0.0309+

PSA: Prostate-specific antigen; GS: Gleason score; RM: resection margin; #Student's *t*-test; *χ^2 test; +Kaplan-Meier method.

were diagnosed with bGS ≥8 prior to surgery. A positive resection margin (RM) was observed in 31.2% of patients. Median tumor volume was 1.88 ml and 17% showed BCR following RP. Median observation period in this study was 63.43 months (Table I).

The prognostic significance of PI-RADS v2.1 scoring system and its relation to patient backgrounds. We divided patients into those with PI-RADS v2.1 score ≤3 and those with ≥4 and compared patient backgrounds between groups (Table II). Patients with a score ≥4 were more likely to be older (p=0.0465), with a higher iPSA level (p=0.0006), more advanced T stage (p=0.0002) and classification as high risk by the D'Amico classification (p=0.0033). A higher incidence of a positive RM was observed in patients with a score ≥ 4 as compared to those with a score of ≤3 (39.5 *vs.* 12.6%, p<0.0001). In addition, higher PI-RADS v2.1 score was correlated positively with larger tumor volume (p<0.0001, Table II and Figure 1A). Median tumor volumes were 0.57 ml, 0.78 ml, 0.73 ml, 1.87 ml, and 4.28 ml for scores 1-5, respectively (Figure 1A). Kaplan–Meier analysis showed shorter progression-free survival (PFS) in patients with PI-RADS v2.1 score 5 than those with ≤4 (p<0.0001, Figure 1B). Furthermore, to moderate the difference in patients' backgrounds, the propensity score-matching (PSM) method was employed for evaluating the prognostic value. After 1:1 PSM based on age at operation, initial PSA, and bGS, a total of 112 patients, 56 each, were considered. Patients with preoperative PI-RADSv2.1 score 5 had unfavorable outcomes as compared to those with ≤4 (p=0.0366, Figure 1C).

In addition, we investigated a prognostic significance of PI-RADS v2.1 score 5 using Cox proportional hazard models and found that iPSA [hazard ratio (HR)=1.88, p=0.0291], PSA density (HR=1.09, p=0.0008), clinical T stage (≥2c) (HR=4.16, p<0.0001), bGS (≥8) (HR=4.37, p<0.0001), and PI-RADS v2.1 score 5 (HR=4.12, p<0.0001) were associated with PFS in univariate analyses (Table III). After multivariate analysis, clinical T stage [HR=2.32, 95% confidence interval (CI)=1.23-4.38, p=0.0093], bGS (HR=2.81, 95%CI=1.55-5.1, p=0.0007), and PI-RADS v2.1 (HR=2.24, 95%CI=1.19-4.23, p=0.0124) were found to be independent prognostic factors for PFS (Table III).

Prognostic value of the D'Amico classification and PI-RADS v2.1 scoring system. Based on the prognostic importance of PI-RADS v2.1 score, we hypothesized that PI-RADS v2.1 could improve the capability to predict clinical outcomes of existing risk classifications (*e.g.*, D'Amico classification). We confirmed that the D'Amico classification system

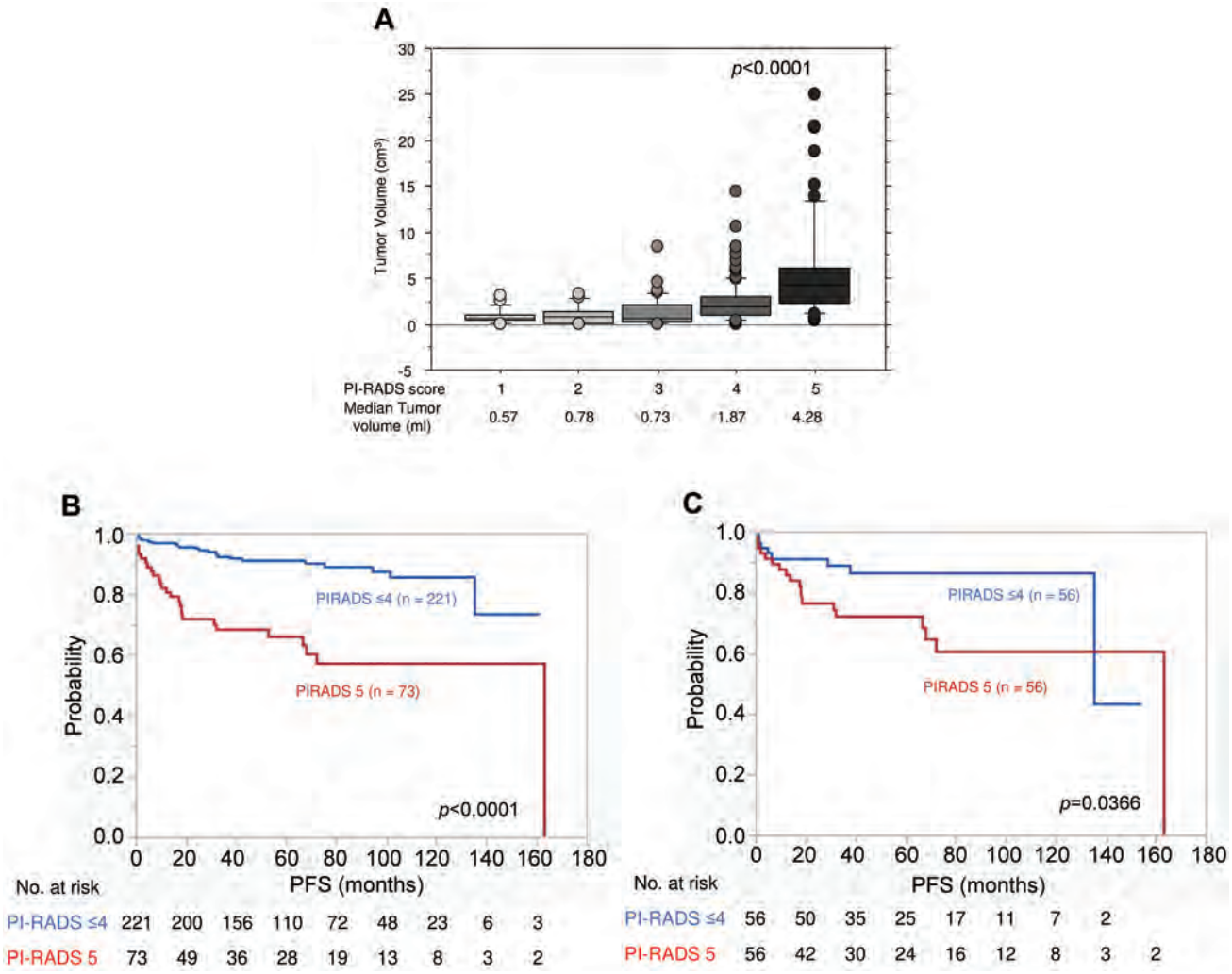

Figure 1. *Prognostic significance of the PI-RADS v2.1 score in patients who underwent radical prostatectomy. (A) Tumor volumes calculated from prostatectomy specimens at each PI-RADS score. (B) Kaplan–Meier analysis classified by the PI-RADS score ≤4 vs. 5 for progression-free survival (PFS). (C) Kaplan–Meier analysis classified by the PI-RADS score ≤4 vs. 5 for PFS after propensity score-matching.*

Table III. *Uni- and multivariate cox proportional hazard models for progression-free survival.*

	Univariate			Multivariate		
	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Age (≥67)	1.21	0.69-2.15	0.5065	-	-	-
Initial PSA (≥7.68)	1.88	1.07-3.42	0.0291	1.11	0.56-2.21	0.7587
Prostate volume (≥29 ml)	1.09	0.61-1.92	0.7741	-	-	-
PSAD	2.79	1.51-5.51	0.0008	1.62	0.77-3.41	0.2081
Clinical T stage (≥T2c)	4.16	2.30-7.37	<0.0001	2.32	1.23-4.38	0.0093
Biopsy GS (≥8)	4.37	2.48-7.69	<0.0001	2.81	1.55-5.10	0.0007
PI-RADS v2.1 score 5	4.12	2.36-7.23	<0.0001	2.24	1.19-4.23	0.0124

HR: Hazard ratio; CI: confidence interval; PSA: prostate-specific antigen; PSAD: PSA density; GS: Gleason score; PI-RADS: prostate imaging-reporting and data system.

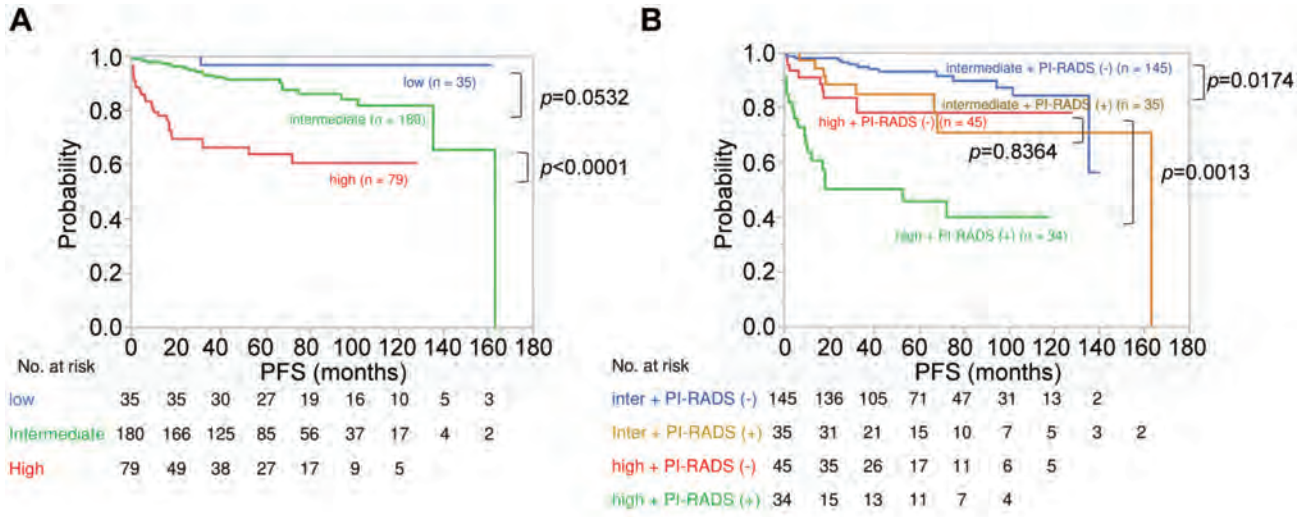

Figure 2. *Validation of the risk classifications for progression-free survival following radical prostatectomy. (A) The D'Amico classification system. (B) Risk classification integrating the D'Amico classification and PI-RADS v2.1 score.*

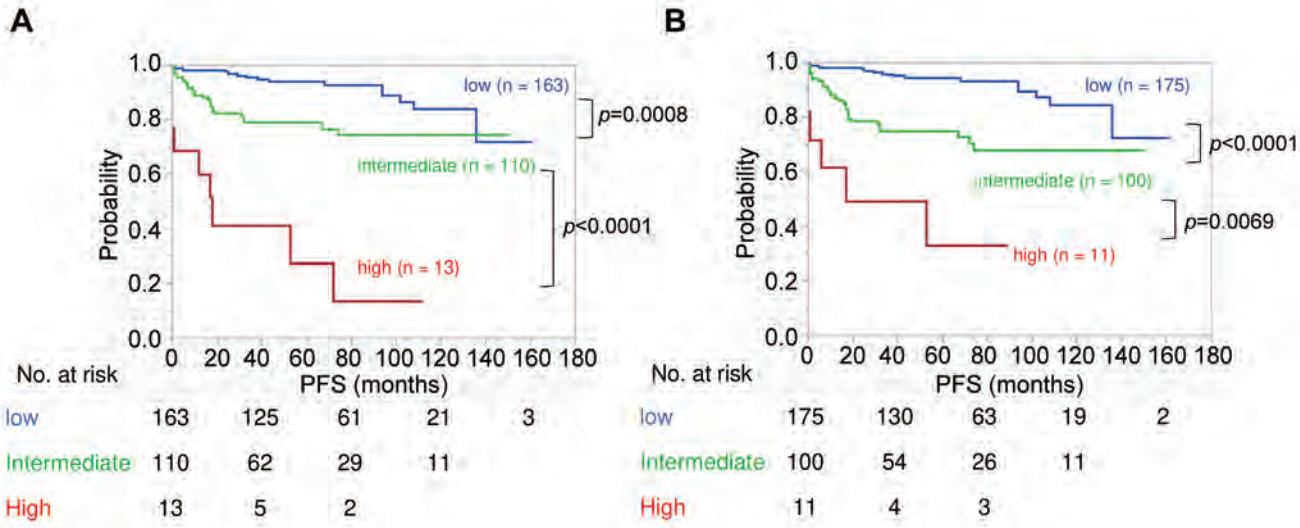

Figure 3. *Prognostic significance of novel risk classifications. (A) A risk classification based on cT ≥2c, bGS ≥8, and PI-RADS 5. (B) A risk classification based on PSA ≥7.68, bGS ≥8, and PI-RADS 5.*

including clinical T stage, PSA level, and bGS stratified patient prognosis in our cohort (Figure 2A). Patients with the D'Amico high-risk had shorter PFS than those with intermediate risk (p<0.0001, Figure 2A). Of note, PI-RADS v2.1 score 5 significantly differentiated prognosis in both intermediate- and high-risk groups in the D'Amico classification (p=0.0174 and p=0.0013, respectively, Figure 2B). Moreover, patients with intermediate-risk and PI-RADS v2.1 score 5 showed comparable prognosis as compared to those with high-risk and PI-RADS v2.1 score ≤4 with 84.8% and 78.1% in 5-year PFS rate (p=0.8364, Figure 2B). HR (high *vs*. others) of the risk classification was improved from 4.74 to 7.2 when combined PI-RADS v2.1 score to the D'Amico classification (Table IV). This analysis indicated the importance of PI-RADS v2.1 score in addition to the D'Amico risk classification for better risk classification when considering treatment options.

Novel risk classification to predict BCR following RP. We further proposed a novel risk classification comprising clinical

Table IV. *Comparison of the capability of risk classifications to predict progression-free survival.*

	Univariate		
	HR	95% CI	*p*-Value
D'Amico classification	4.74	2.68-8.49	<0.0001
D'Amico + PI-RADS v2.1 classification	7.2	3.92-12.83	<0.0001
Our risk classification (cT, bGS, PI-RADS v2.1)	9.71	4.38-19.35	<0.0001
Our risk classification (PSA, bGS, PI-RADS v2.1)	7.03	2.66-15.5	0.0004

HR: Hazard ratio; CI: confidence interval; PSA: prostate-specific antigen; GS: Gleason score; PI-RADS: prostate imaging-reporting and data system.

T stage (≥2c), bGS (≥8), and PI-RADS v2.1 score from multivariate analysis (Table III). This novel classification was defined as low (met 0 factors), intermediate (met 1 or 2 factors), or high (met 3 factors) and Kaplan–Meier analysis showed significant difference between these three groups (low *vs*. intermediate: $p=0.0008$; intermediate *vs*. high: $p<0.0001$) (Figure 3A). The HR for the high-risk group compared to others was 9.71 (95%CI=4.38-19.35) (Table IV).

In addition, we established another risk classification including PSA (≥7.68 ng/ml), bGS (≥8), and PI-RADS v2.1 score, since PI-RADS score basically reflects cancer size and might have a positive correlation with clinical T stage. Risk classification was similarly defined, and significant differences were observed between low-, intermediate-, and high-risk groups ($p<0.0001$ and $p=0.0069$, respectively) (Figure 3B). The HR was 7.03 (95%CI=2.66-15.5) (Table IV). Thus, our risk classification identified a patient population with extremely poor prognosis, achieving a higher HR than the conventional classification.

Discussion

Our study revealed the prognostic importance of the preoperative PI-RADS v2.1 scoring system in patients who had undergone RP. Higher PI-RADS score was associated with increased tumor volumes in the RP specimens. Furthermore, novel risk classifications integrating PI-RADS v2.1 score and the D'Amico classification were developed to improve prognostic capability in comparison with the D'Amico classification alone.

Previous studies have focused on how this scoring system could improve the accuracy of targeted prostate biopsy and avoid unnecessary biopsies (7, 14-16). Positive biopsy rates were significantly improved by fusion prostate biopsy using PI-RADS 4 and 5 lesions as compared to systematic biopsy (64% *vs*. 42.9%, respectively; $p=0.035$) although inclusion of PI-RADS 3 did not improve rates, indicating that PI-RADS ≥4 lesions should be recommended for prostate biopsy (16). Wang *et al*. established a nomogram including bpMRI PI-RADS v2.1 to minimize unnecessary biopsies (7). They found that a predictive model based on bpMRI could improve the ability of clinically significant prostate cancer detection and bpMRI score was correlated strongly with Gleason grade group (7). These findings indicated the clinical utility of PI-RADS scoring system to determine the relevance of performing prostate biopsies.

In addition, several studies have shown that pre-biopsy PI-RADS score could predict clinical outcomes following definitive local treatment (17, 18). A systematic review and meta-analysis revealed that higher PI-RADS v2 classifications were correlated with an increased risk of BCR after local treatment (18). Gandaglia *et al*. investigated 804 patients who received prostate biopsies and developed a risk classification for predicting PSA failure following RP (19). A predictive model including PI-RADS v2 score achieved the highest accuracy to predict clinically significant prostate cancer for identifying patient populations harboring a higher risk of early recurrence after operation (19). Furthermore, a recent study proposed simplified PI-RADS (S-PI-RADS) that is based on bi-parametric MRI (bpMRI) and is easier to use in clinical practice (20). S-PI-RADS has been found to enhance the detection and diagnosis of PCa as well as local recurrence following radiotherapy and RP (20). These results suggested a prognostic role for PI-RADS v2 classification in addition to diagnosis prior to prostate biopsies (18). However, few reports have examined the prognostic significance of PI-RADS v2.1 since the revision in 2019.

The D'Amico classification system has emerged in 1998 and has become one of the most widely used modalities for risk assessment of localized prostate cancer (8). The system is based on tumor stage by serum PSA level, Gleason grade, and clinical T-score, and divides patients into low-, intermediate-, and high-risk groups to evaluate the probability of recurrence (8). These risk groups have been employed in determining the duration of androgen deprivation therapy (ADT) when radiation therapy is administered (21-23). Furthermore, this risk classification has also been used to determine whether and to what extent

lymph node dissection should be performed, and to determine the course of treatment for RP (24, 25). Mandel *et al*. studied the rationale of lymph node dissection among the D'Amico intermediate-risk patients, and found that the detection rate for lymph node metastasis was low among patients with GS ≤6, cT ≤2b, PSA 10-20 ng/ml, indicating that lymph node dissection may not be necessary in some intermediate-risk cases (25). Thus, this classification is an indicator that serves as a basis for making treatment decisions in localized prostate cancer.

However, this classification has never been modified, and its ambiguity is sometimes noted. In particular, the clinical T stage classification has been obscure in that the diagnostic method is not defined as digital rectal examination or MRI image finding (26). In addition, there is a difference between the National Comprehensive Cancer Network (NCCN) guidelines and the D'Amico classification, *e.g.*, cT2c is classified as an intermediate risk in the NCCN guidelines, however, as high risk in the D'Amico classification system. Our findings showed that PI-RADS scoring was significantly correlated with tumor volume. Based on these results, we hypothesized that the PI-RADS scoring system might offer an alternative implement to the cT stage. Our novel risk classification including PI-RADS score, bGS, and PSA level achieved higher HRs than the D'Amico classification. Furthermore, given that the PI-RADS scoring system is an objective indicator and can be clearly scored, it might be a better clinical biomarker of tumor progression in combination with PSA level and bGS.

In conclusion, we indicated the prognostic significance of the PI-RADS v2.1 scoring system in patients who had undergone RP. The PI-RADS v2.1 scoring system can further stratify patient prognosis in the D'Amico classification and can be incorporated into risk stratification schemes to improve the precision of predicting patient prognosis. Our current exploration might help the decision-making for treatment and post-treatment follow-up in patients with localized prostate cancer who underwent definitive treatment.

Conflicts of Interest

The Authors declare that there are no conflicts of interest in relation to this study.

Authors' Contributions

Conceptualization: Y.F., Y.Y., S.S.; Methodology: Y.F., Y.Y., T.H., X.Z., K.S., S.N., Y.K., H.S., Y.G., T.S., Y.I.; Supervision: M.K., A.F., S.S., T.U., T.I.; Writing – original draft: Y.F.; Writing – review & editing: Y.Y., S.S.

Acknowledgements

The present study was supported by the KAKENHI grant 23K19497 and Translational Research Grant of Urological Oncology.

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Received October 14, 2023
Revised November 10, 2023
Accepted November 13, 2023

Anticancer Res. 2024 Feb;44(2):639-647. doi: 10.21873/anticanres.16853.

Copy Number Gain in Androgen Receptors Predicts the Poor Prognosis in Japanese Castration-resistant Prostate Cancer

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PMID: 38307556 DOI: 10.21873/anticanres.16853

Abstract

Background/aim: The prognostic significance of androgen receptor amplification (AR amp) in cell-free DNA (cfDNA) was studied in Japanese patients with castration-resistant prostate cancer (CRPC).

Patients and methods: A total of 120 serum samples were obtained from 38 patients with CRPC. Serum cfDNA was purified and the AR copy number was determined. Factors associated with progression-free survival (PFS) and overall survival (OS) were statistically investigated.

Results: The number of patients administered enzalutamide (Enza)/abiraterone (Abi)/docetaxel (DTX) was 33/25/11, respectively. The median PSA was 16.5 ng/ml. Thirty patients (79%) had bone metastases and three patients (7.9%) had lung metastases. The median follow-up was 655 days. The median initial AR copy number was 1.27 (1.10-11.50); an AR copy number of 1.27 or higher was defined as an AR-amp. Regarding PFS, the presence of AR-amp, Gleason score (GS), and ALP were significant factors in univariate analysis. In multivariate analysis, AR amplification was an independent prognostic factor (hazard ratio=7.7, p=0.0035). For OS, PSA and AR-amp were significant factors. In multivariate analysis, AR-amp (hazard ratio=4.65, p=0.0188) was the only independent prognostic factor.

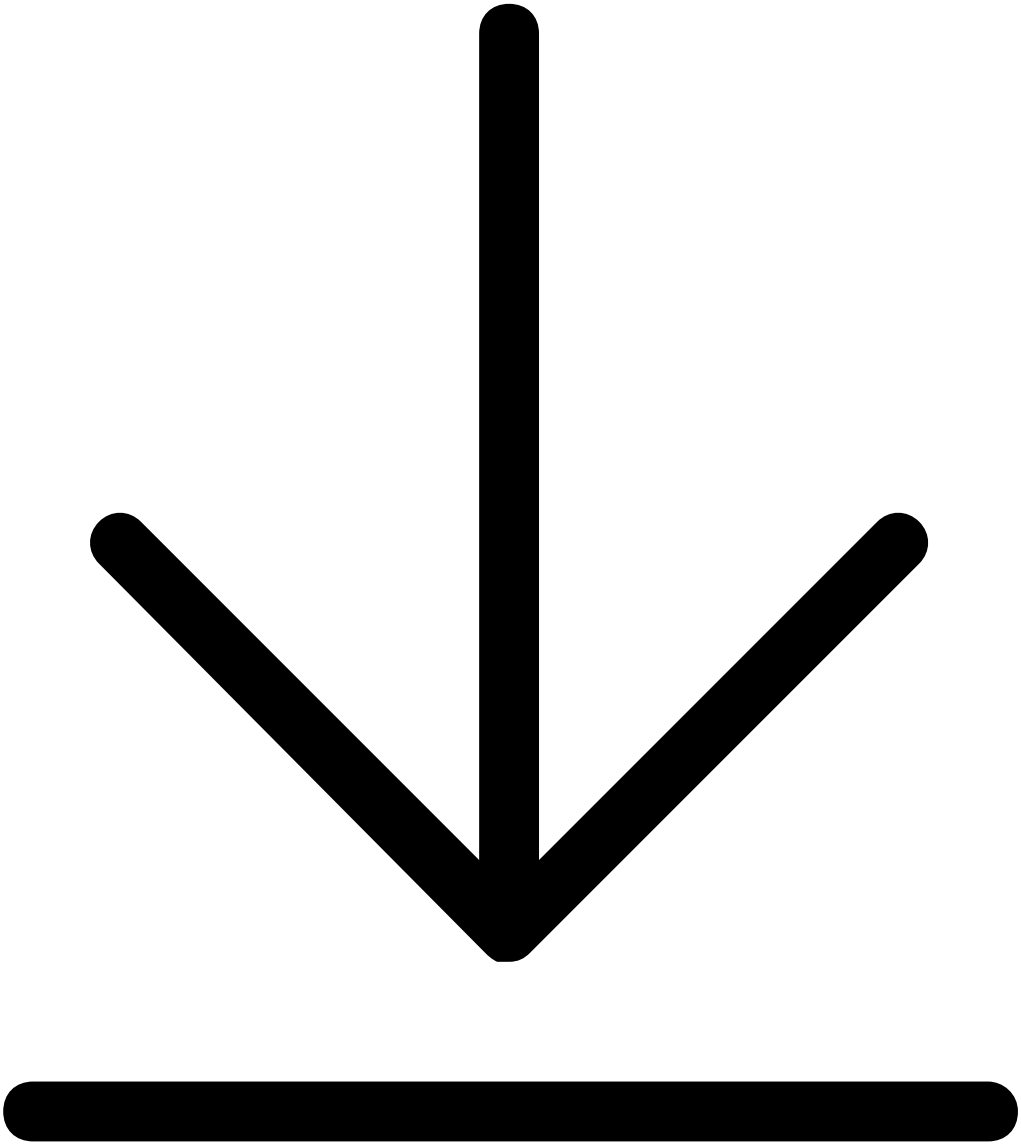
Conclusion: AR-amp was associated with high nadir PSA and low iPSA/PSA ratio. AR-amp was significantly associated with poor prognosis in Japanese patients with CRPC.

Keywords: Cell-free DNA; biochemical recurrence; castration-resistant prostate cancer; liquid biopsy; prognosis.

NCI CPTC Antibody Characterization Program

Miscellaneous

NCI CPTAC Assay Portal



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日中笹川医学奨学金制度(学位取得コース)評価書
課程博士：指導教官用

第 43 期　　研究者番号：G4303　　作成日　：2023 年 3 月 10 日

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専攻種別	□論文博士		☑課程博士		

研究者評価（指導教官記入欄）

成績状況	優　良　可　不可 学業成績係数=	取得単位数 12／12
学生本人が行った研究の概要	姚氏は、修士論文の研究を発展させ、博士後期課程において「在留中国人高齢者の老いへの準備教育プログラムの開発－ビデオカンファレンスを活用して－」と題する研究に取り組んだ。開発した教育プログラムは、在留中国人高齢者の要介護生活の送り方に関する認識の広がりや老後の生活の方向性の明確化を促すなど要介護生活に焦点を当てた老いへの準備に有用であること、また、ビデオカンファレンスを活用したプログラムは便利で参加しやすく、参加者の経験を共有しつつ多様な意見を交換できたことから、実現可能であることが確認できた。看護援助が十分に行き届かない在留中国人高齢者に対して、ビデオカンファレンスという新たな教育方法を用いた老いへの準備性を高めるプログラム開発は新規性があり、今後の普及が期待できる研究であると高く評価された。	
総合評価	【良かった点】 姚氏は、積極的に博士研究を遂行し、規程の 3 年間でやり遂げた。その過程で 3 本の論文を執筆・投稿している。また、研究能力向上のための研修を自ら探して受講したり、中国語と日本語に堪能であることからオリンピックの通訳なども担った。向上心を持ち、何事にも果敢にチャレンジする積極性は群を抜いている。明るい性格で、後輩育成に尽力するなど人望も厚い。 【改善すべき点】 様々なことにチャレンジするため、提出期限が定められているものに対して、ギリギリになることもある。好奇心旺盛であることは評価できるので、それを維持しつつ、期限を意識した計画的な遂行ができるとなお良いと思われる。 【今後の展望】 大学教員として必要な教育・研究能力を高め、博士課程で培った能力を人材育成と研究の発展に思う存分発揮してほしい。また、看護学分野での中国と日本の架け橋となり、両国の親交に寄与することを期待する。	
学位取得見込	2023 年 3 月に博士（看護学）の学位を取得予定。	
評価者（正木治恵）		

日中笹川医学奨学金制度(学位取得コース)報告書
研究者用

第43期　　研究者番号：G4303　　作成日：2023年3月8日

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研究テーマ	在留中国人高齢者の老いへの準備教育プログラムの開発 ービデオカンファレンスを活用してー Development of an education program on aging-related preparation through videoconference for old Chinese migrants in Japan				
専攻種別	論文博士	□	課程博士	☑	

１．研究概要（1）

1)研究背景と目的

在留外国人高齢者の増加に伴い、要介護リスクが高い在留中国人高齢者も増えている。言語の障壁で日本の介護保険制度の認知度が低いことに加え、母国に介護保険制度はなく、高齢者の介護は外部の支援を得ず家庭内で担うといった文化の違いがある。そのため、要介護在留中国人高齢者及びその家族は、介護保険サービスの理解や利用に困難がある。老いに伴う「母国語がえり」現象（日本語の忘れが多く、母国語での会話が増えること）によって、専門職との意思疎通はさらに困難である。加えて、介護サービスの提供側も文化・言語の障壁があるため、外国人高齢者のニーズや意思の把握に困難を抱えている。そして、これから要介護生活を迎える在留中国人高齢者およびその家族が、いざとなったとき困難に陥らず自分が望んだ要介護生活や介護に関する意思を専門職に伝え、適時に専門的な支援を得るための準備が必要であると考えた。しかしながら、将来のケアニーズの予測困難、ケアに関する資源・情報の欠如、計画を組み立てる自信がないなどが将来のケアニーズの準備に影響を与えると報告されている。これらのことから、要介護生活に焦点を当てた老いへの準備教育支援が必要と考える。よって、本研究の目的は、ビデオカンファレンスを活用して要介護生活に焦点を当てる老いへの準備教育プログラムの開発とした。

2)用語の定義

(1)在留中国人高齢者

本研究では、中長期在留資格を持つ日本に住んでいる65歳以上の中国国籍を持つ者、日本の国籍を取得した華僑・華人と、長年に中国で生活していた中国残留邦人とする。

(2)要介護生活に焦点を当てる老いへの準備性

加齢とともに心身が衰えることに起因した要介護状態に対して、介護が必要となる心身的健康状態・生活状態への理解や態度、社会資源の知識、要介護生活の準備に関する認識および自己意思表出の能力などを指す。

3)戦略と方法

本研究は以下の２段階に構成された。

【研究1】要介護生活に焦点を当てた老いへの準備教育プログラムの作成

(1)研究1-1:教育プログラム原案の設計

システマティック・レビューを通して、中国国内および海外に在住する中国人高齢者の施設及び自宅での療養生活のあり様と課題を明らかにし、プログラム原案の内容を作成した。また、ジェロゴジー理論（高齢者の学習理論）を基盤として、教育プログラの実施形態を設計し、教育プログラム原案を作成した。

(2)研究1-2: 教育プログラム原案の妥当性の検討

勤務経験5年以上の看護学研究者3名と訪問看護師1名、ケアマネジャー1名に教育プログラム原案の資料（講義の動画と冊子）及び評価アンケートを送り、アンケートに回答した結果を踏まえて専門家会議を実施し、教育プログラム原案の妥当性を検討した。

【研究2】作成した教育プログラムの実現可能性と有用性の検討

この段階の研究デザインはpre-post testを用いた混合的研究法の説明的順次デザインであった。有用性は介入前後に中国語版の老いへの態度尺度（The Attitudes To Aging Questionnaire：AAQ）及び将来のケアニーズへの準備尺度(Preparation for Future Care Needs Scale-14：PFCN-14)の回答を通して検討した。実現可能性は介入中に各回授業の評価アンケートを回答した結果を踏まえて、参加経験に関するインタビューの実施を通して検討した。
研究実施にあたり、千葉大学大学院看護学研究院倫理審査委員会の承認を得ていた（承認番号：RN4-13、RN4-26）。
データ分析は単純集計と質的帰納的分析を実施した。

4)結果

(1)対象者の基本属性

本研究は首都圏のA県とB県、関西のC県から7組（14名）の研究対象者を募集した。夫婦関係のペアが6組であり、母と娘の親子関係のペアが1組であった。性別について、男性が6名で、女性が8名であった。年齢は53歳から84歳であり、平均年齢は74.9歳であった。在日年数は4年から40年であり、平均在日年数は27.1年であった。

(2)参加経験の全体分析結果

個々対象者が語った参加経験の個別分析で得られた79枚の最終ラベルから、4段階の集めを経て、6つのカテゴリーが生成しされた。これらは【支援が得られる老後の生活環境を確かめた安心感】【健康で充実な日々と不確定な将来によって抑えられた老いへの準備姿勢】【老いや老後の生活に関する認識の広がりと啓発】【授業中に起こった老後の生活を備える認識や行動の変容】【オンライン授業のアクセスしやすさ】【自立と持病状況に影響された自己健康評価】であった。

（次のページに続く）

(3)各回授業アンケートの分析結果
14名の対象者から6章分の授業評価アンケートを計84部（100%）回収した。アンケートは選択項目と自由記載欄を設けた。選択項目について、「４回目：今回の授業を通して、自宅や施設の介護サービスの利用を始めたい状況を述べるようになった」を「当てはまらない」と評価した対象者は1名であり、「授業中の音声の聞きやすさ」を「当てはまらない」と評価した対象者は２名があった。その他の評価項目はすべて「大体当てはまる」「かなり当てはまる」「非常に当てはまる」と評価された。自由記載欄のコメントは、質的帰納的分析を経て、次の6つのカテゴリーが生成された。これらは、「オンライン授業を参加するメリットと不便」「講義内容は実用性があって自分のニーズに満たした」「要介護生活への関心が高くなり、考えられるようになった」「将来の生活の予測困難」「講義内容と資料は多様性があって、わかりやすかった」「ビデオの活用と議論を加えることで、講義が豊かになり、講義内容をより深く理解でき、イメージしやすくなった」であった。

(4)介入前後のAAQ及びPFCN-14の変化
14名の対象者からAAQ尺度とPFCN-14尺度は介入前後にわたって、計28部（100%）を回収した。その結果、介入後にAAQとPFCN-14の総点数が両方とも高くなった対象者は４名（28.6%）であり、いずれが高くなった対象者は９名（64.3%）であった。つまり、13名（92.9%）の対象者に老いへの態度や将来のケアニーズへの準備性の向上にポジティブな効果があった。また、９名（62.3%）の対象者に将来のケアニーズへの準備性の向上にポジティブな効果が見られた。AAQとPFCN-14尺度の各ドメインの変化について、AAQの心理的獲得ドメイン（78.6%）とPFCN-14の意識ドメイン（85.7%）にポジティブな変化が最も多かった。しかし、１名(7.1%)の対象者は老いへの態度および将来のケアニーズへの準備性にポジティブな効果が見えなかった。

5)考察
【オンライン授業のアクセスしやすさ】「オンライン授業を参加するメリットと不便」という参加者の参加経験と授業評価のコメントがあった。Chenら(2016)は、高齢者に対してICTの活用は、社会とのつながり、興味がある活動の参加、ソーシャルサポートを得ることに効果があると報告している。在留中国人高齢者は文化や言語の違いから地域で開催される支援プログラムに参加しにくい上に、地域で中国人高齢者向け講座は少なく、各地域に散住している彼らを１つの地域に集めることは困難である。そこで、中国人高齢者が社会や他者とのつながる重要な手段となっているICTの活用が有効と考え、ビデオカンファレンスを活用して教育プログラムを開催した。ビデオカンファレンスの活用は、便利かつアクセスしやすく、高齢者にとって外出疲労の軽減や地域での支援を得にくい現状の改善に貢献できるため、在留中国人高齢学習者に良い学び場の提供に新規性があると考えられる。
一方、各回授業評価アンケートでは、「ビデオの活用と議論を加えることで、講義が豊かになり、講義内容をより深く理解でき、イメージしやすくなった」というコメントが多く見られた。ジェロゴジーという高齢者学習援助理論では、ライフスパンに渡って重ねてきた経験は高齢者の学習に貢献できるため、高齢者の人生経験を活用できる学習セッションを設ける必要性が述べられている(John, 1988)。本研究はジェロゴジー理論を基盤として教育プログラムの実施形態を検討し開発した。教育プログラムの実施では、講義内容をスライドに示しながら、講義内容を伝わるビデオや写真を活用した。また、講義後に参加者間の議論も設けた。議論では老後の生活と老いの経験について多様な意見を交換でき、要介護生活の理解を深めることと認識の広がりを促した。その理由は、在留中国人高齢者にとって、これから迎えに来る要介護生活は未経験であり、周りに参考となるケースも少ない。議論では、同じ背景がある参加者が講義内容と自身の状況に合わせて個々の意見や経験を共有し、現実的な事例を提供していた。対象者は議論を通して、今経験している高齢期の生活及び将来の要介護生活の計画に参考となる新たなアイデアや情報を入手でき、講義内容を深く理解することに促しうると言える。また、講義中のビデオや写真の活用は、授業中に挙げられた事例をリアルに示され、授業内容のイメージしやすさと理解の深さにつながった。よって、本研究で開発した教育プログラムは在留中国人高齢者にとって実現可能であり、ジェロゴジー理論は中国人高齢者にとって効果的な学習援助方法であると考えられる。
毎回事業評価アンケートの回収結果では、授業内容を「実用性があって自分のニーズに満たした」「将来に役に立つ」と高く評価された。また、彼らが語った参加経験から【支援が得られる老後の生活環境を確かめた安心感】というカテゴリーが生成された。堀(2012)は、60代より70代以上の高齢者は「老後」や「老後の生き方」に関する内容の学習要求率が比較的に高いと報告している。本研究に参加した14名の対象者の平均年齢は74.９歳であり、そのうち、後期高齢者は11名であった。教育プログラムの内容は要介護生活に焦点を当てた学習であり、老いの自覚症状を経験している対象者自身の状況に強く関連し、在留中国人高齢者の学習ニーズに満たし、将来、日本での老後の生活を安心して送ることに寄与できると言える。
介入後のAAQやPFCN-14のいずれかがポジティブになった対象者は9割弱であり、老いへの準備意識が強まった対象者は8割強だった。また、彼らの参加経験では、【老いや老後の生活に関する認識の広がりと啓発】【授業中に起こった老後の生活を備える認識や行動の変容】というカテゴリーがあった。春日(2018)は「老いの支度」を高齢期で生じる様々なリスクを最小限にとどめるために、まだ判断力や自己決定力がある元気な間に、必要な福祉や医療・介護に関する制度的知識や情報収集、対処方法を学び、暮らしのあり方や人間関係を組み替え、自分自身の将来のために自ら備える活動であると定義し、さらに、高齢者はその支度に着手する必要性を主張している。しかし、一般的な高齢者はその支度について、何をどのように備えるのかはわからないと述べられている。本研究で開発した教育プログラムの内容は老いに伴う変化、自宅及び施設での療養生活のあり様、日本における介護保険制度、老いへの準備の必要性、要介護生活の計画が含まれた。教育プログラムの参加を通して、老後の生活の変移、要介護生活のイメージ付き及びその準備の方向性が明瞭になったから、対象者の将来のケアニーズの準備性の向上に見られたと言える。これらのことから、本研究で開発した教育プログラムは一般市民向けの講座として開催し、地域に在住する元気な中国人高齢者の生涯学習に期待できる。また、保健や福祉に関する行政や医療の専門職にとって、在留中国人高齢者の老いへの準備の支援策の検討に貢献できると考えられる。

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１．研究概要（2）
1)研究題名
　在留中国人高齢者の健康に関する思い　-中国人が集まる地域活動に参加する対象者に焦点を当てて-　（投稿論文一原著）

2)目的
　中国人が集まる地域活動に参加する在留中国人高齢者を対象に異国在住での健康に関わる経験の調査を通して,彼らの健康に関する思いを明らかにすることである。

2）用語の定義
(1)在留中国人高齢者：中長期在留資格を持ち日本に住む65歳以上の中国国籍を持つ人,日本の国籍を取得した華僑,華人と,中国で生まれ50年以上中国で生活していた中国残留孤児とする。
(2)健康に関する思い：日本に住んでいることの影響を含めた,自分自身の身体的・精神的・社会的健康及びそれに関連する受診や健康増進について感じること・希望すること・気にかけて考えを持つことやその内容とする。

3）研究方法
(1)対象者および募集方法
　対象者は,本研究で定義する在留中国人高齢者とした。ただし,認知機能障害を持つ者は除外した。
首都圏都市部にある公民館およびコミュニティーセンター,中国語対応が可能な施設で対象者を募集した。また,対象者の多様性を確保するため,雪だるま式募集方法を併用した。
(2)データ収集期間
　2019年3月～10月
(3)調査方法
　調査はインタビューガイドを用いて対象者に半構造化面接を１～３回,１回60分を目安に行った。面接内容は,対象者の同意を得てICレコーダーに録音し,逐語録を作成した。個別面接で使用した言語は対象者の希望に沿い,中国語とした。
(4)調査内容
　調査内容は対象者の基礎情報と健康に関する思いや考えとした。先行研究を参考に,インタビューガイドを作成した。基礎情報は年齢,収入,家族構成,学歴,在留年数とした。
　インタビューガイドの内容は,在留外国人の健康阻害要因と健康に関する異文化体験に関する先行研究(Jiang, 2016; 中嶋, 2015; 平野, 2003)を参考に,在留期間中の受診経験, 日常生活の過ごし方,健康増進方法,今後の生活や健康上の心配や希望,日本語の自己評価理由についてどのような考えや思いを持っているかを含めた。
(5)分析手法
　対象者の基礎情報と日本語能力の自己評価は記述的統計学で分析した。面接データは質的帰納的に分析した。本研究の対象者は社会的・文化的背景に強く影響を受け,個別性が高いと考え,個々の事例が持つ個性・独自性を把握できる質的統合法（KJ法）（山浦, 2012)を参考にした。

4）倫理的配慮
　本研究は,千葉大学大学院看護学研究科倫理審査委員会の承認と対象者が活用する組織や施設の承認を得て実施した（承認番号30-97）。

5）結果
(1)対象者の概要
　本研究の対象者は男性５名,女性８名の計13名であった。対象者全員が首都圏在住であった。在留年数は,５年から30年以上であり,平均22.8年であった。10名の対象者は日本の病院を受診した経験があった。対象者は生活支援金や中国あるいは日本の年金で生活を送っていた。日本語能力の自己評価について,日常会話,医療会話が「まあ良い」と評価した対象者はそれぞれ５名と７名であったが,日本人の友人を持ち,主に日本人で構成されたメンバーでの活動に定期的に参加する人は１名だけだった。対象者は全員中国人が集まる活動や中国人向け地域活動に参加していた。
(2)健康に関する思い
　全体分析結果は,テーマを【　】,サブテーマを〈　〉で示す。全体分析結果は80枚の各個別分析の最終ラベルを素材とし,類似性に沿って統合し,以下の８つのテーマ,16のサブテーマを生成した。
【身体機能や精神状態から主観的に健康の良し悪しを評価している】
　これは健康状態の評価に関する思いであり,〈病弱による良くない自己健康評価〉と〈老いを感じながらも良い自己健康評価〉が含まれた。
【安全で便利な社会に住むことを安心だと感じている】
　これは自分にとって,困難がなく医療機関を利用でき,安全で便利な社会環境に住んでいることに安心しているという〈自分にとって安心できる住む環境〉に関する思いであった。

（次のページに続く）

【他者の支援を受けることで安心して生活できている】
これは,他者の支援に関する思いであり,〈支援された生活〉,〈助けを求める生活〉と〈助けを求める対象がいない大変さ〉が含まれた。
【老いを受け止めて前向きに生きている】
これは老いの受容に関する思いであり,〈余生への希望〉と〈前向きな生き方〉を含んでいた。
【言葉の壁で生活は制限されたが中国人との関わりや趣味を通して気楽に生活している】
これは,言葉の壁があり日本人とのコミュニケーションが難しいことによる制限された生活の中で,中国人との関わりや趣味を通して,社会とのつながりを作り,気楽に生活しているという,〈制限された生活の中での楽しさ〉に関する思いであった。
【家族で互いに支える生活を継続したい】
これは家族関係への思いであり,〈子供に迷惑をかけたくない〉と〈家族の支え〉を含んでいた。
【良い医療サービスをうまく利用できるので安心している】
これは,医療サービスの利用に関する思いであり,〈心配のない医療サービス〉と〈言語の壁に影響された受診〉を含んでいた。
【健康を維持するため中医学に基づき自主的に健康管理をしている】
これは,中医学に基づいた健康管理に関する思いであり,〈健康情報の入手〉〈中医学に基づく健康促進〉と〈総合的な健康管理〉を含んでいた。

6)考察
本研究では,中国人が集まる地域活動に参加する在留中国人高齢者に焦点を当てた8つの健康に関する思いを明らかにした。高齢期の発達課題や,言葉による制限,中国文化に影響された思いが含まれていた。本研究は,高齢期の発達課題や,言語による制限,儒教思想や中医学の影響から対象者の特徴を考察する。

(1)高齢期の発達課題に影響した健康に関する思い
【身体機能や精神状態から主観的に健康の良し悪しを評価している】【安全で便利な社会に住むことを安心だと感じている】【他者の支援を受けることで安心して生活できている】【老いを受け止めて前向きに生きている】という思いは高齢期に直面する健康や生活の変化に関連していた。
対象者は健康上の変化に適応するために身体・精神面の状態をとらえると同時に,生活上の支援や居心地のよい便利な住まい環境を整えていた。対象者らは高齢期における変化への対応を踏まえて,加齢に伴う現状を受容していたと考える。健康の衰退に適応し,生活を満足におくれるように(住まいを)準備することや,自分の人生の受容は高齢期の発達課題として挙げられている。本研究の対象者は健康の衰退およびそれによって変化した現状への適応は,異国での老後の生活の中で,高齢期の発達課題に直面しつつ発達し続けていると考えられる。

(2)言葉による制限が影響した健康に関する思い
【言葉の壁で生活は制限されたが中国人との関わりや趣味を通して気楽に生活している】【良い医療サービスをうまく利用できるので安心している】という思いは言葉の制限が社会活動の参加と医療機関の利用に関連したことを示した。
本研究の対象者が居住する首都圏都市部では,在留中国人高齢者が多く,中国人が自発的に集まる活動や外国人向けの活動がさかんであった。そこでは交通が便利で,高齢者に対する交通費の公的な補助制度があるため,中国人が集まる活動にアクセスしやすいと考える。一方,本研究の対象者は,老いに伴う身体機能や記憶力の衰えによって外出が制限され,日本語を忘れることが増えた実感から,今後も療養生活を自宅で送ることを希望していた。今後自宅で療養生活を送る在留中国人高齢者が増えると推測されるため,医療・福祉分野における専門的通訳ボランティアの養成やICTを活用して遠方から通訳を受ける仕組みづくりなど,医療サービスにアクセスしやすい環境の整備が必要であると考える。

(3)儒教思想や中医学に影響される健康に関する思い
【家族で互いに支える生活を継続したい】【健康を維持するため中医学に基づき自主的に健康管理をしている】という思いは中国文化に影響していた。
本研究の対象者の健康に関する思いには,子は親の面倒を見るべきであり,できる限り健康に生きることで子供の負担を軽減しようとする親としてのあり方が反映された中国の伝統的な親子関係が継承されており,それは対象者の老後生活の希望,健康を維持する意欲につながっていたと考える。また、在留中国人高齢者では,移住後の生活においても中医学の影響が継続しており,ケアを提供する際は,健康への対処方法に中医学が根づいていることを理解する必要がある。中医学の健康促進方法の特徴や効果を考慮して食生活や運動習慣などにケアに活かすことは,彼らにとって馴染みがある継続可能な支援となると考える。

7)結論
在留中国人高齢者においては,高齢期の発達課題や,中国文化,言葉の壁が彼らの健康に関する思いに影響を与えていることが明らかとなった。言葉による制限や母国文化の継続は彼らの安心感や,健康促進,老後生活の希望に強く影響を及ぼすため,言葉の壁を取り除く環境整備や彼らに馴染んだ文化や健康促進方法に基づいたケアの提供の重要性が示唆された。

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(4)山浦晴男 (2012). 質的統合法入門 : 考え方と手順. 医学書院.

2. 執筆論文 Publication of thesis ※記載した論文を添付してください。Attach all of the papers listed below.

論文名 1 Title	在留中国人高齢者の健康に関する思い ― 中国人が集まる地域活動に参加する対象者に焦点を当てて ―					
掲載誌名 Published journal	文化看護学会誌(原著)					
	2022 年 5 月	14(1) 巻(号)	21 頁 ～ 30 頁	言語 Language	日本語	
第1著者名 First author	姚利	第2著者名 Second author	石井優香	第3著者名 Third author	山崎由利亜	
その他著者名 Other authors	石橋みゆき,正木治恵					
論文名 2 Title	日本に長期在住する中国人高齢者の健康管理―地域で自立した生活を送る1事例の語りより―					
掲載誌名 Published journal	日中医学(一般投稿)					
	2023 年 2 月	37(4) 巻(号)	26 頁 ～ 32 頁	言語 Language	日本語/中国語	
第1著者名 First author	姚利	第2著者名 Second author	石井優香	第3著者名 Third author	正木治恵	
その他著者名 Other authors	無					
論文名 3 Title	Older Chinese people's experiences of relocation to long-term care facilities: A literature review of qualitative studies					
掲載誌名 Published journal	Journal of International Nursing Research(査読中)					
	年 月	巻(号)	頁 ～ 頁	言語 Language	英語	
第1著者名 First author	Li Yao	第2著者名 Second author	Harue Masaki	第3著者名 Third author	無	
その他著者名 Other authors	無					
論文名 4 Title						
掲載誌名 Published journal						
	年 月	巻(号)	頁 ～ 頁	言語 Language		
第1著者名 First author		第2著者名 Second author		第3著者名 Third author		
その他著者名 Other authors						
論文名 5 Title						
掲載誌名 Published journal						
	年 月	巻(号)	頁 ～ 頁	言語 Language		
第1著者名 First author		第2著者名 Second author		第3著者名 Third author		
その他著者名 Other authors						

3. 学会発表 Conference presentation ※筆頭演者として総会・国際学会を含む主な学会で発表したものを記載してくだ

※Describe your presentation as the principal presenter in major academic meetings including general meetings or international me

学会名 Conference	World Academy of Nursing Science The 7th International Nursing Research Conference of WANS				
演 題 Topic	Chinese older adults' perspective of home care: A systematic review of qualitative studies(査読あり)				
開催日 date	2022 年 10 月 18～19 日	開催地 venue	Taiwan		
形式 method	□ 口頭発表 Oral ☑ ポスター発表 Poster	言語 Language	□ 日本語 ☑ 英語 □ 中国語		
共同演者名 Co-presenter	Yang Huiching; Masaki Harue; Zhou Wei				
学会名 Conference	第14回文化看護学会学術集会				
演 題 Topic	中国人高齢者の施設での療養生活に関する認識 —文献検討を通して—(査読あり)				
開催日 date	2022 年 3 月 12 日	開催地 venue	栃木県下野市		
形式 method	☑ 口頭発表 Oral □ ポスター発表 Poster	言語 Language	☑ 日本語 □ 英語 □ 中国語		
共同演者名 Co-presenter	正木治恵				
学会名 Conference	日本老年看護学会 第28回学術集会 合同開催 第33回 日本老年学会総会				
演 題 Topic	在留中国人高齢者の老いへの準備教育プログラムの有用性の検討(採択)(査読あり)				
開催日 date	2023 年 6 月 16～18 日	開催地 venue	日本・横浜		
形式 method	☑ 口頭発表 Oral □ ポスター発表 Poster	言語 Language	☑ 日本語 □ 英語 □ 中国語		
共同演者名 Co-presenter	正木治恵、呉小玉				
学会名 Conference	International Association of Gerontology & Geriatrics(IAGG) Asia/Oceania Regional Congress 2023				
演 題 Topic	Verification of the feasibility of an education program on aging-related preparation through videoconferences for old Chinese migrants in Japan(査読中)				
開催日 date	2023 年 6 月 12～15 日	開催地 venue	Japan/Yokohama		
形式 method	☑ 口頭発表 Oral □ ポスター発表 Poster	言語 Language	□ 日本語 ☑ 英語 □ 中国語		
共同演者名 Co-presenter	Masaki Harue				

4. 受賞(研究業績) Award (Research achievement)

名 称 Award name				
	国名 Country		受賞年 Year of	年 月
名 称 Award name				
	国名 Country		受賞年 Year of	年 月

5. 本研究テーマに関わる他の研究助成金受給 Other research grants concerned with your resarch theme

受給実績 Receipt record	■ 有　□ 無
助成機関名称 Funding agency	文化看護学会
助成金名称 Grant name	2021年度文化看護学会研究助成金
受給期間 Supported period	2022 年 1 月 ～ 2024 年 12 月
受給額 Amount received	100,000 円
受給実績 Receipt record	■ 有　□ 無
助成機関名称 Funding agency	公益財団法人 在宅医療助成 勇美記念財団
助成金名称 Grant name	2021年度在宅医療助成（後期）一般公募「在宅医療研究への助成」
受給期間 Supported period	2022 年 3 月 ～ 2024 年 3 月
受給額 Amount received	885,396 円

6. 他の奨学金受給 Another awarded scholarship

受給実績 Receipt record	□ 有　■ 無
助成機関名称 Funding agency	
奨学金名称 Scholarship name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円

7. 研究活動に関する報道発表 Press release concerned with your research activities

※記載した記事を添付してください。Attach a copy of the article described below

報道発表 Press release	□ 有　■ 無	発表年月日 Date of release	
発表機関 Released medium			
発表形式 Release method	・新聞 ・雑誌 ・Web site ・記者発表 ・その他（ ）		
発表タイトル Released title			

8. 本研究テーマに関する特許出願予定 Patent application concerned with your research theme

出願予定 Scheduled	□ 有　■ 無	出願国 Application	
出願内容（概要） Application contents			

9. その他 Others

指導責任者（記名） 正木治恵

原著論文

在留中国人高齢者の健康に関する思い
― 中国人が集まる地域活動に参加する対象者に焦点を当てて ―

Health Perspective of Elderly Chinese Migrants in Japan: Focus on Participants of Chinese Attracted Community Activities

姚　利[1)]，石井優香[1)]，山崎由利亜[1)]，石橋みゆき[2)]，正木治恵[2)]

Li Yao, Yuka Ishii, Yuria Yamasaki, Miyuki Ishibashi, Harue Masaki

キーワード：主観的健康感，健康に関する思い，高齢者，在留中国人，文化看護

Key words：self-rated health, health perspective, elderly, Chinese migrant, Transcultural Nursing

Abstract

Purpose

To investigate the health perspectives regarding to self-rated health and health experiences of elderly Chinese migrants who were involved in Chinese attracted community activities in Japan.

Method

Participants were 13 elderly Chinese residents who were recruited in the Tokyo Area; individual semi-structured interviews and qualitative and inductive analyses were performed.

Results

From the results of the overall analysis, eight health perspectives were identified: (1) subjectively examining health according to physical and mental faculties; (2) feeling secure by living in a safe and amenity social environment; (3) feeling peaceful by accepting support from others to solve daily life issues; (4) keeping a positive frame of mind by accepting the realities of aging; (5) communicating and engaging with other Chinese residents, so that daily life is not restricted by the language barriers; (6) preferring to reside with family members and support each other; (7) feeling relieved to be able to access Japanese healthcare services successfully by themselves; and (8) independently using traditional Chinese medicine to manage health and keep fit.

Conclusion

We confirmed that late adulthood developmental tasks, Chinese culture, and language barriers affected the health perspectives of elderly Chinese migrants in Japan. Because the language barriers and continuity of the native culture influenced their security, health promotion, and expectation of later life, it is important to create

受付日：2021年９月30日
採択日：2022年３月９日
１）千葉大学大学院看護学研究科　Doctoral Program, Graduate School of Nursing; Chiba University
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an accommodating environment to resolve language barriers and provide care based on the cultural and health promotion standards that are familiar to elderly Chinese migrants.

要　旨

目　的

中国人が集まる地域活動に参加する在留中国人高齢者を対象に異国在住での主観的健康感と健康に関わる経験の調査を通して，彼らの健康に関する思いを明らかにすることである。

方　法

日本の首都圏に在住する中国人高齢者13名を対象に個別に半構造化面接を実施し，質的帰納的に分析した。

結　果

全体分析により，【身体機能や精神状態から主観的に健康の良し悪しを評価している】【安全で便利な社会に住むことを安心だと感じている】【他者の支援を受けることで安心して生活できている】【老いを受け止めて前向きに生きている】【言葉の壁で生活は制限されたが中国人との関わりや趣味を通して気楽に生活している】【家族で互いに支える生活を継続したい】【良い医療サービスをうまく利用できるので安心している】【健康を維持するため中医学に基づき自主的に健康管理をしている】という８つの健康に関する思いが明らかとなった。

結　論

在留中国人高齢者においては，高齢期の発達課題や，中国文化，言葉の壁が彼らの健康に関する思いに影響を与えていることが明らかとなった。言葉による制限や母国文化の継続は彼らの安心感や，健康促進，老後生活の希望に強く影響を及ぼすため，言葉の壁を取り除く環境整備や彼らに馴染んだ文化や健康促進方法に基づいたケアの提供の重要性が示唆された。

Ⅰ．背　景

2020年末時点で65歳以上の在留外国人の総数は197,197人に達し，2019年より0.7％増え，日本全人口の6.7％を占めている。そのうち，中国人高齢者は23,080人（11.7％）であり，在留外国人高齢者総数の第２位である（出入国在留管理庁，2021）。彼らは，1978年の日中国交正常化および1980年代後半の外国人労働力の受け入れ政策によって，就職するために来日し，現在，高齢期を迎えている。また，2012年には，高度外国人材の優遇措置の一つとして「親の帯同」を許可し始め（法務省入国管理局，2012），母国にいる高齢の親を日本に呼び寄せることが少なくない。そのため，現在の在留中国人高齢者には，中国で生まれ中国文化の中で長年過ごし，来日した人が多いと思われる。

先行研究では，外国人患者の看護提供において，言葉（近藤，2021），文化，病気に関する考え方の違い（久保，2014）による困難が多く報告されている。今後，外国人患者への看護ケア提供の機会の増加に伴い，ケア提供上の困難も増えることが推察される。一方，在日外国人にとって，保健医療機関を利用する際の言葉の壁・異なる文化や価値観の壁・異文化不適応から生じた悩みの解消の壁は，彼らの健康問題を及ぼす要因である（呉，2016）と指摘されている。つまり，日本に移住することによる言葉や文化の壁は，在留外国人の健康に影響を及ぼしていると考えられる。

Leininger（1995）は，看護の対象となる多くの人々に健康と安寧をもたらすために文化に適したケアの提供を目標とし，多様な文化を理解することが不可欠であることを提唱している。高齢者ケアでは，高齢者を支えている内在化した「文化」を十分に理解した上で，ケアに生かすことが重要である（正木，2004）。在留中国人高齢者が，日本で高齢期の変化を経験しつつ心身ともに健康で安寧に暮らし続けるために，看護師は彼らが持っている文化，価値観や習慣を理解し，文化を考慮したケアを提供する必要があると考えられる。しかし，異国で老いを経験している中国人高齢者

の健康に対する内在化された中国文化の影響と，移住したことの影響は明らかになっていない。

一方，個々の生活様式や価値観が多様化してきた現在，主観的健康感などの，個人レベルでみた主観的な健康指標が重視されている（小田，2007）。芳賀（1984）は主観的健康感は身体的，精神的，社会的な統合体としての健康の主観的認識を表していると示唆している。主観的健康感を１つの指標として，中国の文化が内在化した在留中国人高齢者の健康状態及び健康に関する思いを理解できるのではないかと考える。本研究は，在留中国人高齢者の主観的健康感と日本での健康に関わる経験を明らかにすることで，より適切なケア提供への示唆が得られると考える。また，各地域に散住している在留中国人高齢者にアクセスするため，中国人が集まる地域活動に参加する対象者に焦点を当てた。

Ⅱ．研究目的

中国人が集まる地域活動に参加する在留中国人高齢者を対象に異国在住での主観的健康感と健康に関わる経験の調査を通して，彼らの健康に関する思いを明らかにすることである。

Ⅲ．用語の定義

在留中国人高齢者：中長期在留資格を持ち日本に住む65歳以上の中国国籍を持つ人，日本の国籍を取得した華僑，華人と，中国で生まれ50年以上中国で生活していた中国残留孤児とする。

主観的健康感（Self-Rated Health）：生活機能の状態や疾病の有無にかかわらず，自分自身が自己の健康状態をどのようにとらえるかを評価するもの（大内，2010）とする。

健康に関する思い：日本に住んでいることの影響を含めた，自分自身の身体的・精神的・社会的健康及びそれに関連する受診や健康増進について感じること・希望すること・気にかけて考えを持つことやその内容とする。

Ⅳ．研究方法

１．対象者および募集方法

対象者は，本研究で定義する在留中国人高齢者とした。ただし，認知機能障害を持つ者は除外した。

首都圏都市部にある公民館およびコミュニティーセンター，中国語対応が可能な施設で対象者を募集した。また，対象者の多様性を確保するため，雪だるま式募集方法を併用した。

２．データ収集期間

2019年３月～10月

３．調査方法

調査はインタビューガイドを用いて対象者に半構造化面接を１～３回，１回60分を目安に行った。面接内容は，対象者の同意を得てICレコーダーに録音し，逐語録を作成した。個別面接で使用した言語は対象者の希望に沿い，中国語とした。

４．調査内容

調査内容は対象者の基礎情報と主観的健康感，健康に関する思いや考えとした。先行研究を参考に，インタビューガイドを作成した。

基礎情報は年齢，収入，家族構成，学歴，在留年数とした。

主観的健康感は健康に関する思いの１つの指標として調査を行い，その測定方法は杉澤ら（1995）が明らかにした測定方法を参考に，「自分の健康状態を全体的にどう評価しますか」「他の同年齢の方と比べて自分の健康をどう評価しますか」の問いに，「とても良い」から「良くない」まで４段階での回答を求めた。また，「去年の自分の状態と比較すると，現在の健康状態をどう評価しますか」の問いに対しては，「いつも通り」を含めた５段階での回答を求めた。

インタビューガイドの内容は，在留外国人の健康阻害要因と健康に関する異文化体験に関する先行研究（Jiang, 2016；中嶋，2015；平野，2003）を参考に，在留期間中の受診経験，日常生活の過ごし方，健康増進方法，今後の生活や健康上の心配や希望，主観的健康感の評価理由および日本語の自己評価理由についてどのような考えや思いを持っているかを含めた。日常会話および受診時の言葉の壁は在留外国人の精神的健康・主観的健康感に影響を及ぼす（大植，2018）ため，日本語の自己評価についても「日本語の日常会話能力をどう評価しますか」と「日本語の医療会話能力をどう評価しますか」の問いに，「とても良い」から「良くない」まで４段階での回答を求めた。

５．分析手法

対象者の基礎情報，主観的健康感，日本語能力の自己評価は記述的統計学で分析した。面接データは質的

帰納的に分析した。本研究の対象者は社会的・文化的背景に強く影響を受け，個別性が高いと考え，個々の事例が持つ個性・独自性を把握できる質的統合法（KJ法）（山浦，2012）を参考にした。

個別分析は，面接の逐語録から一つの「健康に関する思い」が含まれるようにラベルを作成し，ラベル内容の類似性に沿ってグループを編成し表札を作成した。最終ラベルが５～７枚になるまで同じ作業を繰り返した。

全体分析は，個別分析の最終ラベルを内容の類似性に沿って集め，サブテーマをつけた。さらにサブテーマを類似性に沿って集め，テーマとした。ただし，類似する内容がないサブテーマはテーマとした。

６．信憑性

研究者は質的統合法（KJ法）研修会へ参加し，分析手法の精度の向上に努めた。分析の全過程において老年看護学ならびに質的統合法（KJ法）に精通する研究者のスーパーバイズを受けた。

収集したデータはすべて中国語であった。日本語でスーパーバイズを受けて分析方法の妥当性を確保するため，最初の５名の個別分析は中国語から日本語に訳したあと行った。訳したデータの妥当性を確保するため，研究者の所属する大学の日本語支援室に添削を依頼した。言語のニュアンスを確保するために，６人目からは中国語で分析を行った。ただし，最終ラベルは日本語と中国語で作成し，指導教員のスーパーバイズを受けた。

Ⅴ．倫理的配慮

本研究は，千葉大学大学院看護学研究科倫理審査委員会の承認と対象者が活用する組織や施設の承認を得て実施した（承認番号30-97）。

対象者に対して研究の目的と参加の自由，個人情報の保護方法，研究に参加しない場合であっても不利益を受けないこと，研究結果の公表の可能性などの倫理事項について分かりやすい言葉と文章で説明し，書面にて同意を得られた後に面接を実施した。

Ⅵ．結　　果

１．対象者の概要

本研究の対象者は男性５名，女性８名の計13名であった（表１）。対象者全員が首都圏在住であった。在留年数は，5年から30年以上であり，平均22.8年であった。10名の対象者は日本の病院を受診した経験があった。対象者は生活支援金や中国あるいは日本の年金で生活を送っていた。日本語能力の自己評価について，日常会話，医療会話が「まあ良い」と評価した対象者はそれぞれ５名と７名であったが，日本人の友人を持ち，主に日本人で構成されたメンバーでの活動に定期的に参加する人は１名だけだった。対象者は全員中国人が集まる活動や中国人向け地域活動に参加していた。

各対象者の総面接時間は30分から140分であり，平均時間は50分であった。個別分析に用いた元ラベルは40枚から80枚であった。

２．研究対象者が活用する組織や施設の概要

１）X県健康教室

X県健康教室は中国残留邦人と在留中国人が日本の社会に入れるように，日本文化を学び，健康知識や日本語能力を高めるため，自発的に立ち上げられた組織である。活動の頻度は週１回である。およそ35名の参加者は全員70歳以上である。

２）X県日本語サークル

X県都市部の公民館で週１回，近隣に住む中国人に，日常会話能力の向上と日本の文化の学習を目的として，日本語授業を開催している。参加者12名のうち，70歳以上の中国人高齢者は４名である。

３）Y県デイサービス

中国残留邦人や中国人高齢者に介護サービスを提供するデイサービスである。サービス内容は中華風の食事や入浴，健康チェック，機能訓練，中国の昔のゲームなどである。職員は看護師１名，生活相談員６名，調理人１名である。生活相談員は全員介護職経験が長く，日本語が堪能な中国人や中国残留邦人２世である。看護師は日本人で中国語を翻訳する機器を用い，コミュニケーションを取っている。

４）その他

１）－３）以外の地域活動を参加する対象者は雪だるま式を通して募集した。これらの活動は，公園など公的な場所で近所の中国人が自発的に集まり，活動の内容・参加人数・参加時間などは明確に決められておらず自由に参加できる。

３．全体分析結果

１）主観的健康感

主観的健康感の結果を表２に示す。全体的評価，同年齢者との比較に対し，「とても良い」及び「まあ良

表1　対象者概要（n＝13）

	性別	年齢	学歴	在留年数（年）	在留資格	世帯状況	収入	日本語能力の自己評価		ADL	募集方法
								日常会話	医療会話		
A氏	男	60代後半	高校	5－9	永住	同	年金	良くない	良くない	自立	雪
B氏	女	70代前半	小学校	30－34	永住	同	生活支援金	良くない	良くない	自立	健
C氏	女	60代後半	大学	25－29	永住	独	年金	まあ良い	まあ良い	自立	雪
D氏	男	70代後半	大学	30－34	永住	同	年金	良くない	まあ良い	自立	健
E氏	女	70代後半	中学校	20－24	定住	独	生活支援金	良くない	まあ良い	自立	日
F氏	女	70代後半	中学校	30－34	定住	同	生活支援金	良くない	まあ良い	要介護	デイ
G氏	男	60代後半	高校	5－9	特定活動	同	年金	良くない	良くない	自立	雪
H氏	女	70代後半	大学	35－39	日籍華人	同	年金	まあ良い	まあ良い	自立	健
I氏	女	70代前半	無	20－24	永住	独	年金	まあ良い	良くない	自立	健
J氏	男	60代後半	高校	5－9	特定活動	同	年金	良くない	良くない	自立	雪
K氏	女	60代後半	高校	20－24	永住	同	自営業収入	まあ良い	まあ良い	自立	雪
L氏	女	70代後半	無	25－29	永住	同	生活支援金	良くない	良くない	自立	健
M氏	男	70代後半	大学	35－39	定住	同	年金	まあ良い	まあ良い	自立	健

※特定活動：法務大臣が個々の外国人について特に指定する活動
※定住者：法務大臣が特別な理由を考慮し一定の在留期間を指定して居住を認める者。残留孤児を含む
※日籍華人：日本の国籍を取得した中国系住民
※永住者：法務大臣が永住を認める者
※同：同居
※独：独居
※雪：Z県雪だるま式で募集
※健：X県健康教室
※日：X県日本語サークル
※デイ：Y県デイサービス

表2　主観的健康感（n＝13）

	とても良い	まあ良い	いつも通り	あまり良くない	良くない
全体的評価	5(39%)	6(46%)		2(15%)	0(0%)
同年齢者と比較	4(31%)	8(61%)		0(0%)	1(8%)
去年と比較	1(8%)	3(23%)	5(38%)	1(8%)	3(23%)

い」と評価したのはそれぞれ11名（85％），12名（92％）であった。去年との比較に対し，「とても良い」及び「まあ良い」と評価したのはそれぞれ１名（８％），３名（23％）であった。

２）健康に関する思い

全体分析結果は，テーマを【　】，サブテーマを〈　〉，研究対象者の語りの内容を斜字で示す。全体分析結果は80枚の各個別分析の最終ラベルを素材とし，類似性に沿って統合し，以下の８つのテーマ，16のサブテーマを生成した。

(1)【身体機能や精神状態から主観的に健康の良し悪しを評価している】

これは健康状態の評価に関する思いであり，〈病弱による良くない自己健康評価〉と〈老いを感じながらも良い自己健康評価〉が含まれた。〈病弱による良くない自己健康評価〉は，重病や自立して生活できないことで健康状態が良くないと思っていることだった。〈老いを感じながらも良い自己健康評価〉は，老いを感じているが，体や精神の状態が良いから，健康状態は相対的に良いと思っていることだった。

(2)【安全で便利な社会に住むことを安心だと感じている】

これは自分にとって，困難がなく医療機関を利用でき，安全で便利な社会環境に住んでいることに安心しているという〈自分にとって安心できる住む環境〉に関する思いであった。

日本の食品は天然で体には無害，加えて空気はきれいだし，安心できる（A氏）。

（私は）近所に中国語が対応できる病院があり，（高齢者が）無料で乗れる電車の駅の近く，南向きで静かな部屋に引っ越した。外出と受診が便利だから（B氏）。

(3)【他者の支援を受けることで安心して生活できている】

これは，他者の支援に関する思いであり，〈支援された生活〉，〈助けを求める生活〉と〈助けを求める対象がいない大変さ〉が含まれた。〈支援された生活〉

は，日本の行政機関の生活支援を受けながら安心して暮らしていることだった。〈助けを求める生活〉は，困難を取り除くために周りの人や行政機関に助けを求めることだった。一方で，〈助けを求める対象がいない大変さ〉について，C氏は，*自分は独身で，将来認知症や他の病気で自立して生活できなくなったら，介護保険を利用して支援を得るが，介護保険に認定されない期間に急に病気になって面倒を見てくれる人がいない状況になることを心配していると語った。*

(4)【老いを受け止めて前向きに生きている】

これは老いの受容に関する思いであり，〈余生への希望〉と〈前向きな生き方〉を含んでいた。

〈余生への希望〉は人生の最期は死であり，老いに伴う身体機能の衰え，社会の役割の制限等の現状を受け止めて，思うように生きたいという思いだった。

人生の最期は死なので，生活に希望や心配はないが，寝たきりや虚弱になり，不自由や苦しみを味わい，他の人に面倒をかけることが嫌なので，最期はコロリと逝きたい（D氏）。

〈前向きな生き方〉は加齢や疾病などの現状を受け止めて，前向きに生きているという思いだった。

人間の生死は運命であり，楽あれば苦ありを信じていて，毎日後悔なく楽しんで大事に過ごせば，人生は有意義になる（E氏）。

(5)【言葉の壁で生活は制限されたが中国人との関わりや趣味を通して気楽に生活している】

これは，言葉の壁があり日本人とのコミュニケーションが難しいことによる制限された生活の中で，中国人との関わりや趣味を通して，社会とのつながりを作り，気楽に生活しているという，〈制限された生活の中での楽しさ〉に関する思いであった。

（中国人向けの）デイーサービスでは中国語で活動に参加し友達と雑談することが楽しい（F氏）。

違う言語や文化（の影響）で日本人とのコミュニケーションができないから，朝中国人が集まる公園で太極拳を見たり，中国人の床屋さんで散髪したりして，のんびりと過ごしている（A氏）。

年をとって記憶力が衰えるため，日本語を覚えるより忘れる方が多い……楽しく，有意義な生活を過ごすため，毎日外出して（中国人の）友達と歓談したり，互いに助けあったり，優しい言葉をかけて人間関係を維持している（E氏）。

(6)【家族で互いに支える生活を継続したい】

これは家族関係への思いであり，〈子供に迷惑をかけたくない〉と〈家族の支え〉を含んでいた。

〈子供に迷惑をかけたくない〉は老後は子供に迷惑をかけないように過ごしたいということだった。

一家団欒の方が安心だと思い，将来の老後の生活は，（病気になると子供に迷惑をかけるから）子供に迷惑をかけず健康で（子供と）同居生活を送りたい（G氏）。

今後私は寝たきりになって（自分で）病院にも行けなくなり，娘も仕事があるから，迷惑をかけたくないし。もし仕方がないなら，老人ホームに行くしかない…でも私は本当に老人ホームに入りたくない，自分の家がいい（H氏）。

〈家族の支え〉は家族の支えで悩みがなく暮らしていることだった。

夫が亡くなった後，（私に）寂しさを感じさせないように孫が同居してくれて，日常生活に悩みがある時，娘が相談にのってくれるし，孝行な家族に支えられて，悩みや辛い時期を乗り越えた。日常生活に解決できないことはない（I氏）。

(7)【良い医療サービスをうまく利用できるので安心している】

これは，医療サービスの利用に関する思いであり，〈心配のない医療サービス〉と〈言語の壁に影響された受診〉を含んでいた。

〈心配のない医療サービス〉は医療サービスを心配なく利用できるので安心を感じるという思いだった。

年を取ったら必ず病気をすると思うが，病気になる事を心配しながらも日本の医者は責任を持って薬を処方してくれるし，自分も中国と日本の健康保険があるから，病気になっても経済的な心配がなく，安心して速やかに受診できるので，病気になっても怖くない（J氏）。

〈言語の壁に影響された受診〉は，日本語がわからない対象者の受診の困難さであった。一方で，日本語が概ねわかる人にとって，あるいはよく受けている医療行為であれば，一人で受診する自信があること，対応できない場合でも家族や役所の通訳者に同伴してもらい，中国語が対応できる医療機関を利用することであった。

日本語は大体わかるので，いつも受診や入院している主治医や病院があり，救急車を呼んだ経験もあるし，自分一人で受診や入院することに問題はない（F氏）。

入院期間中や受診時の悩みは，子供に迷惑をかけな

いように自分で解決し……遠い病院に行くときや受診時に日本語が分からないなど自分で解決できない場合は夫と娘に同伴してもらった（H氏）。

(8)【健康を維持するため中医学に基づき自主的に健康管理をしている】

これは，中医学に基づいた健康管理に関する思いであり，〈健康情報の入手〉〈中医学に基づく健康促進〉と〈総合的な健康管理〉を含んでいた。その中でも，老いを感じたため中医学に基づく多様な方法で健康を促進しているという〈中医学に基づく健康促進〉についての思いが多かった。

蓮の葉や枸杞を使って調理するという「求人不如求自己」の中医学の本に書かれたコレステロール値をコントロールできる料理を食べたり，毎朝ツボマッサージをして風邪を予防したりしている（I氏）。

長生きのためにカシュウを調理して白髪を予防したり，天然の蜂蜜を食べて免疫力を高めたり，辛い食べ物を減らしたりという健康な飲食習慣を守っている（A氏）。

〈健康情報の入手〉は，健康を管理するため，メディアから中国や日本の健康促進情報を入手することだった。〈総合的な健康管理〉は，健康な体を守るため，自主的に運動や飲食，外出の増加などを通して，身体的，精神的，社会的健康を総合的に管理していることだった。

自分の体を鍛えるため，毎朝公園で太極拳を実践している……毎日家にいることは良くない。家で寝るより活動に参加することが良い。活動を参加した後は，（家にいるより）精神状態が違う（気分が晴れる）（D氏）。

Ⅶ. 考　察

本研究では，中国人が集まる地域活動に参加する在留中国人高齢者に焦点を当てた８つの健康に関する思いを明らかにした。高齢期の発達課題や，言葉による制限，中国文化に影響された思いが含まれていた。本研究は，主観的健康感や，高齢期の発達課題，言語による制限，儒教思想や中医学の影響から対象者の特徴を考察する。

１）主観的健康感

全体的および同年齢者と比較した健康感が良いと評価した対象者が８割以上を占めた。一方，先行研究（胡，2007）では，日本に在住する中国残留孤児の主観的健康感は低かった。この違いの要因は２つ考えられる。

１つ目は，本研究の対象者は定期的に中国人や中国残留孤児が集まる地域活動に参加していた。于ら（于，2019）は，中国都市部に居住する高齢者は付き合い・交流の頻度が高いほど主観的健康感が高かったと明らかにしている。本研究の対象者の主観的健康感は中国人や中国残留孤児が集まる組織活動の参加の楽しさの影響を受けていたと考える。

２つ目は，本研究の対象者は身体機能や精神状態から主観的に健康の良し悪しを評価していた。13名の対象者のうち11名は，健康状態を同年齢者と比べた際，大きな持病がなく，精神状態が良く，さらに自立して生活できるので，自分の健康に自信を持っていた。先行研究では，疾病への罹患，精神的な安定感（五十嵐，2006；山内，2015），慢性的な健康障害や機能障害（Jiang, 2016）は主観的健康感に影響を与えると報告している。本研究の対象者は，老いに伴う体力の低下などを経験していたが，重病がなくADLが高い者が多かった。そのことが，主観的健康感を良いと評価した対象者の割合が高かったことに関連していると考える。

２）高齢期の発達課題に影響した健康に関する思い

【身体機能や精神状態から主観的に健康の良し悪しを評価している】【安全で便利な社会に住むことを安心だと感じている】【他者の支援を受けることで安心して生活できている】【老いを受け止めて前向きに生きている】という思いは高齢期に直面する健康や生活の変化に関連していた。

対象者は健康上の変化に適応するために身体・精神面の状態をとらえると同時に，生活上の支援や居心地のよい便利な住まい環境を整えていた。対象者らは高齢期における変化への対応を踏まえて，加齢に伴う現状を受容していたと考える。健康の衰退に適応し，生活を満足におくれるように（住まいを）準備すること（Havighurst, 1995）や，自分の人生の受容（Newman, 1988）は高齢期の発達課題として挙げられている。本研究の対象者は健康の衰退およびそれによって変化した現状への適応は，異国での老後の生活の中で，高齢期の発達課題に直面しつつ発達し続けていると考えられる。

３）言葉による制限が影響した健康に関する思い

【言葉の壁で生活は制限されたが中国人との関わりや趣味を通して気楽に生活している】【良い医療サー

ビスをうまく利用できるので安心している】という思いは言葉の制限が社会活動の参加と医療機関の利用に関連したことを示した。

社会活動の参加について，現在，日本では，在留外国人が日本社会で孤立しないようにするために，多文化共生の地域づくり活動を通して，地域社会へ参画できる仕組みを整備している（総務省，2020）。本研究の対象者が居住する首都圏都市部では，在留中国人高齢者が多く，中国人が自発的に集まる活動や外国人向けの活動がさかんであった。そこでは交通が便利で，高齢者に対する交通費の公的な補助制度があるため，中国人が集まる活動にアクセスしやすいと考える。彼らは日本人とのコミュニケーションが難しい中で，中国人との関わりや趣味を通して社会とのつながりを持っていた。Maoら（2020）が行った中国系アメリカ人高齢者を対象にした健康行為と文化変容に関する研究では，中国の文化背景を有する海外移住高齢者は，中国の生活行動パタンを保つことや言葉の障壁で，中国人との関わりを好み，地元の人との関わりが乏しく，移住社会に入りにくいことが多いことが示唆された。つまり，同じ文化背景を有する在留中国人同士の交流にアクセスしやすくするため，交通や地域活動の開催・情報の提供を含む環境の整備が必要であると考える。

医療機関の利用について，本研究の対象者は，在日してすぐの頃は医療サービスを利用する際に困難があったと語ったが，現在，受診の困難があると語ったのは13人の対象者のうち１人だけだった。また，在留年数が10年未満の対象者の方が，医療会話と日常会話能力の自己評価について，両方とも良くない傾向があった。対象者からは在留年数が長いほど受診の経験は多いため，よく受けている医療行為は１人で対応できると語られた。そして，健康教室や日本語サークルに参加している対象者は，日常会話や医療会話能力のいずれかを「まあ良い」と自己評価する人が多かった。対象者の日本での在留期間が長いこと，日本語を学ぶことができる地域活動に参加していることは，日本語での受診時の自信につながると考えられる。

一方，本研究の対象者は，老いに伴う身体機能や記憶力の衰えによって外出が制限され，日本語を忘れることが増えた実感から，今後も療養生活を自宅で送ることを希望していた。自分で対応できない場合は，家族や行政機関の通訳者に同伴してもらい，中国語が対応できる医療機関を利用し，家族の手助けを受けられ，公的医療通訳者や中国語が対応できる医療機関にアクセスしやすい地域に在住していることが受診時の言語の障壁を取り除いていた。Luiら（2017）は，イギリスに在住する中国系移民高齢者は言語の障壁で医療サービスの利用に困難はあるが，家族や，慈善組織，公的通訳者はこの困難を乗り越えるための橋わたしの役割をもつことを報告している。今後自宅で療養生活を送る在留中国人高齢者が増えると推測されるため，医療・福祉分野における専門的通訳ボランティアの養成やICTを活用して遠方から通訳を受ける仕組みづくりなど，医療サービスにアクセスしやすい環境の整備が必要であると考える。

４）儒教思想や中医学に影響される健康に関する思い

【家族で互いに支える生活を継続したい】【健康を維持するため中医学に基づき自主的に健康管理をしている】という思いは中国文化に影響していた。

本研究の対象者は，〈家族の支え〉を受けながら異国で生活し，今後も家族に〈迷惑をかけたくない望み〉を抱いており，健康に老後生活を送りたいという思いがあった。黄ら（2010）は中国系移民高齢者は，青年期までに中国で経験した文化や価値観が移住生活の中でも主導的な価値観となり，老いへの態度・経験に影響を与えていると明らかにしている。これは，本結果と一致する。「中華人民共和国の婚姻法」（1985）では，親に孝行することは法的な義務である。中国の伝統的な文化の主流である儒教思想によると，子供は親孝行すべきであり，親の老後生活は自宅で子供が身体的な介助を担うべきで，家族で全員を支え合いながら老後の生活を送るべきと考えられている。対象者の健康に関する思いには，子は親の面倒を見るべきであり，できる限り健康に生きることで子供の負担を軽減しようとする親としてのあり方が反映された中国の伝統的な親子関係が継承されており，それは対象者の老後生活の希望，健康を維持する意欲につながっていたと考える。

健康の維持について，本研究の対象者からは，生理的老化を予防するため，カシュウ（生薬の一つ）を調理して食べたり，ツボマッサージや太極拳をするなど中医学の健康促進方法について多く語られた。中医学は中国の春秋時期から記載が始まり，1991年には，「中医学と西洋医学を同等に重視する」方針が中国の憲法に記された。中医学の「医食同源」（薬膳）の基本的な考え方によると，食事は薬であり，薬としての効能が働く食べ物を食べて健康を促進する。また，太

極拳は中医学の陰陽概念を取り入れた武道や護身術の一つとして，古代中国で始まった。中華人民共和国が誕生した後，国民の健康促進方法として採用され大衆化した。太極拳はゆったりとした動きが筋力の向上や身体バランスの改善に効果的で（胡，2007），転倒リスクの低下や身体機能改善に有用であると示唆されている（Yu, 2012）。つまり，在留中国人高齢者では，移住後の生活においても中医学の影響が継続しており，ケアを提供する際は，健康への対処方法に中医学が根づいていることを理解する必要がある。中医学の健康促進方法の特徴や効果を考慮して食生活や運動習慣などにケアに活かすことは，彼らにとって馴染みがある継続可能な支援となると考える。

Ⅷ. 研究の限界と課題

本研究は，活動の参加中や終了直後にデータ収集を実施したため，結果に影響を与えた可能性がある。また，本研究の対象者は中国人が集まる地域活動へ参加する者を対象者とした。今後は，中国人同士での活動に参加していない対象者を調査する必要があると考える。さらに，在留中国人高齢者に対する内在化した文化を考慮した健康促進方法を検討する必要があると考える。

Ⅸ. 結　論

本研究は，在留中国人高齢者の健康に関する思いを明らかにすることを目的に調査を行い，8つの健康に関する思いを明らかにした。その結果，在留中国人高齢者の健康に関する思いは高齢期の発達課題に影響を受けた一方で，言葉の壁および中国文化に強く影響されており，中国人同士の関わりの中での楽しさや医療サービスをうまく利用できる安心感は言葉の壁を乗り越えることに関連していた。儒教思想や中医学の健康促進方法という母国文化の継続は彼らの老後生活の希望，健康促進に影響を及ぼしていた。そして，言葉の壁を取り除く環境整備や在留中国人高齢者が馴染んだ文化や健康促進方法に基づいたケアの提供の重要性が示唆された。文化に配慮したケアは在留中国人高齢者の生活の効果的な支援につながると考えられる。

謝　辞

本研究にご理解とご協力をいただいた研究参加者の皆様に謹んで御礼申し上げます。本研究は，2019年度千葉看護学会研究支援金支給事業と日中笹川医学奨学金より助成を受け実施し，2020年度千葉大学大学院修士論文を加筆・修正したもので，要旨は2020TWINC台湾国際看護学会学術集会にて発表した。本研究において利益相反は存在しない。

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一般投稿

日本に長期在住する中国人高齢者の健康管理
―地域で自立した生活を送る1事例の語りより―

Health Promotion Narratives of an Older Chinese Migrant Living Independently in a Community in Japan

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【Abstract】
In this case study, we used a semi-structured interview to clarify the health promotion and perspectives of an older Chinese migrant living independently in a community in Japan. The KJ method, a qualitative data synthesis method was used to analyze the data. We found that based on the life attitude of actively problem-solving, Mr. A visited physicians without language barriers, felt comfortable managing his health, and accepted the realities of aging. As a result, he was satisfied with his health and hoped to spend the rest of his life pain-free.

【Key words】
Aged, Case reports, Chinese, Migrant, Health promotion

はじめに

在日中国人永住者は 296,600 人（2021 年末時点）であり、そのうち 65 歳以上の高齢者は 22,885 人に達し、今後も増加していくと予想される[1]。法務省は、外国人との共生社会のビジョン実現に向けて、中長期的課題及び具体的施策を公表しており、高齢の外国人を取り巻く実態・課題把握の不十分さとそれらを踏まえた支援策の検討の必要性を主張している[2]。

高齢者は、加齢に伴い身体機能の低下など老いの自覚症状が増えるため、健康管理への関心が高くなる。また、健康管理の方法は長年の生活体験とともに築かれるものであり、その人の文化背景や生活環境から影響を受けている[3]。移住期間[4]、移住した国の文化や言葉、医療システムの違いなどは、外国からの移住者の健康管理行動において困難が生じる要因であり、これらの要因は心身の健康に影響を及ぼす[5,6]。以上のことから、日本に長期在住する中国人高齢者が直面した健康管理上の課題と対応方法を明らかにすることは重要であり、在日中国人高齢者の健康促進のための支援策検討に寄与することができる。

家高[7]は 1 事例の特殊性と複雑さの解明は重要な諸状況における活動の理解を通して、様々な類似

事例の理解を促すと述べている。また、石川ら[8]は、高齢者が語るライフストーリー中で一般的には健康づくりとして認識されない内容や本人が健康づくりとしてこれまで意識していなかった内容についても、健康づくりとして意味付けられたと述べている。したがって、本研究の目的を日本に長期在住する中国人高齢者1事例の健康管理に関する語りを通して、地域で自立した生活を送る在日中国人高齢者の健康管理と健康管理に関連する考えを明らかにすることとした。

方　法

1. 研究デザイン

本研究は事例研究である。

2. 対象者募集

本研究は地域のコミュニティーセンターで開催されている外国人向けの活動を通して対象者を募集した。

3. データ収集方法

研究者はインタビューガイドを用いた半構造化インタビユーを行い、データを収集した。インタビューガイドは日本における医療機関の受診経験、日常生活の過ごし方、健康増進の方法、今後の生活を含めた健康上の不安や望み、主観的健康感の評価理由などで構成されている。なお、インタビューガイドは外国人住民の健康問題に関する先行研究[5]をもとに作成した。インタビューは対象者居住地域のコミュニティーセンター内にあるプライバシーが確保された静かな個室で行われた。全てのインタビューは研究者によって録音された。

4. データ収集期日

2019年4月X日に行った。

5. データ分析方法

研究者はインタビューの逐語録をデータ源とし、質的統合法（KJ法）[9]を用いた質的分析を行った。まず、研究者は“日常生活の中でどのように健康管理を行っているか”を分析テーマとしてインタビュー逐語録を単位化し、元ラベルを作成した。次に、類似したラベルを集めてグループ化し、集めたラベル群に対してその内容を最もよく表す一文をラベルとして作成した。同じ作業を類似性がなくなるまで繰り返し、最終的に残ったラベルを最終ラベルとした。その後、最終ラベル同士の相互関係を表す空間配置図を作成し、分析テーマに基づいて空間配置図の構造が直観的にわかるシンボルマークをつけ、関係性を叙述化した。シンボルマークは【健康管理に関する要因：その方法】と示した。分析過程において、老年看護学ならびに質的統合法（KJ法）に精通する研究者のスーパーバイズを受けた。

6. 倫理的配慮

本研究は千葉大学大学院看護学研究科倫理審査委員会の承認を得た（承認番号30-97）。対象者に対して研究の目的、個人情報の保護、インタビューを録音すること、研究結果の公表などの倫理事項について中国語で分かりやすい言葉と文章で説明し、書面にて同意を得た後にインタビューを実施した。

結　果

1. 事例の概要

A氏はB県に在住する70代後半の永住在留資格を持つ男性である。A氏はデータ収集時点から32

年前に妻と息子２人と共に家族４人で日本に移住した。中国では大学卒業後に研究者として働き、来日後は技術者として定年まで働いた。現在は、妻の介護をしながら夫婦２人で生活している。A 氏は骨折によって日本の病院を受診した経験はあったが、インタビュー時点では持病はなかった。A 氏は主観的健康感を去年と比べて“悪くなった”が、同年齢者と比べると“とてもいい”と評価した。また、A 氏は中国人向け健康増進教室に１回 / 週の頻度で参加し、地域の日本人及び中国人に対して、中国語と日本語の授業を行っていた。毎朝、公園で太極拳やラジオ体操を行い、毎日、約 8,000 歩以上を歩いていた。

2. 分析結果

インタビュー逐語録から 65 枚の元ラベルが作成され、5 段階のグループ化を経て、6 つの最終ラベルとシンボルマークが生成された。以下本文において、シンボルマークを【　】、最終ラベルを〈　〉、シンボルマークの内容を表している元ラベルを*斜字*にて示した上で、シンボルマークの内容及びそれらの関係性が反映された空間配置図（**図 1**）について説明している。

（1）A 氏の健康管理のシンボルマークの内容

【人生の基本姿勢：入手できるところから適切な解決方法を探し困難を取り除く】

この最終ラベルは〈研究者として、日常生活や健康に問題があった時、メディアや社会資源など手の届く範囲で解決方法を探求し、自分に合う物を判断し、手に入れて困難を取り除く〉であった。

もし（健康情報が）欲しいなら、情報を入手するためにその情報を探す方法を自分で考えるはず。解決方法を自分で考える。…研究に関する仕事をする人（自分）は、分からないままにはしない。分からないなら、自分で解決方法を探す。

【受診への自信：言葉の壁がなく受診できている】

この最終ラベルは〈言葉の壁により日本の病院の受診が難しい人と違い、自分は必要な時に医療用語を自分で調べることができるので言葉の問題はなく、日本での病院受診に自信がある〉であった。

（医師の話は）全部わかったよ。…私は通訳者は 1 回も呼ばなかった。…他の人は言葉（日本語）の問題がある。（彼らは）病気になった時、日本で治療を受けたが、なかなか（病気が）治らなかった。結局中国の病院に行った（中国に戻って受診した）。彼らは言葉（の意味）も通じないし、通訳者も彼らの考えを医師にうまく伝えられなかったからだ。

【健康管理による心地よさ：運動や団体活動の参加が楽しい】

この最終ラベルは〈運動で健康を維持することが当然だと思ったり、授業の準備や団体活動への参加を楽しいと感じたり、自らの健康をきちんと管理している〉であった。

他の人に太極拳を教えるのが私の目的ではない。お金ももらっていない。毎朝、自分ひとりでやる。一緒にやりたい人（日本人）がいたらやるし、誰もいなくても大丈夫。（その理由は）自分の体を鍛えるのが目的だから。…（授業を準備する事は）楽しい。暇を潰せるから。朝から晩まで寝るより良い。

【現実の受容：自分でコントロールできない現実を素直に受け入れる】

この最終ラベルは〈老いに伴う体の衰え、社会役割の喪失、受診の待ち時間が長いことなどに対しては対処方法を探し、どうしても対処出来ない場合、現実を受け入れる〉であった。

今、歯が 19 本しか残ってない。元々は 24 本ある

はず、5本なくなってしまった。…日本の“8020(運動)”によると、私は今1本足りない。…しょうがない、このままでいい。

【健康への自負：老いなどの現実を含め自分の健康には相対的に満足している】

この最終ラベルは〈人生の終点は死である事や老い、自分の経済状況などの現実を受け止めた上で、今の健康状態は、相対的にいいと思っている〉であった。

同年齢の人と比べ（自分の健康状態は）、私のほうがいい。日本人でも、中国人でも、私より健康な人はいない。…身体、心理状態、知識も。私の知識は広い。

【最期に対する希望：不自由や苦しみなく逝きたい】

この最終ラベルは〈人生の終点は死なので、将来の生活に心配はないが、最期は不自由や苦しみなく、他者に迷惑をかけずに逝きたい〉であった。

（将来の生活に）心配はない。人間はどうせ死ぬから。心配してもしなくても、変わらない。体が不自由になってから死ぬより、自由に動ける間に死んだほうがいい。他の人に迷惑をかけるより、今死んだほうがいい。社会に負担をかけないし、自分も苦しくない。

(2) A氏の健康管理の空間配置図（図1）

空間配置図（**図1**）に含まれた6つのシンボルマーク【健康管理に関する要因：その方法】の関係

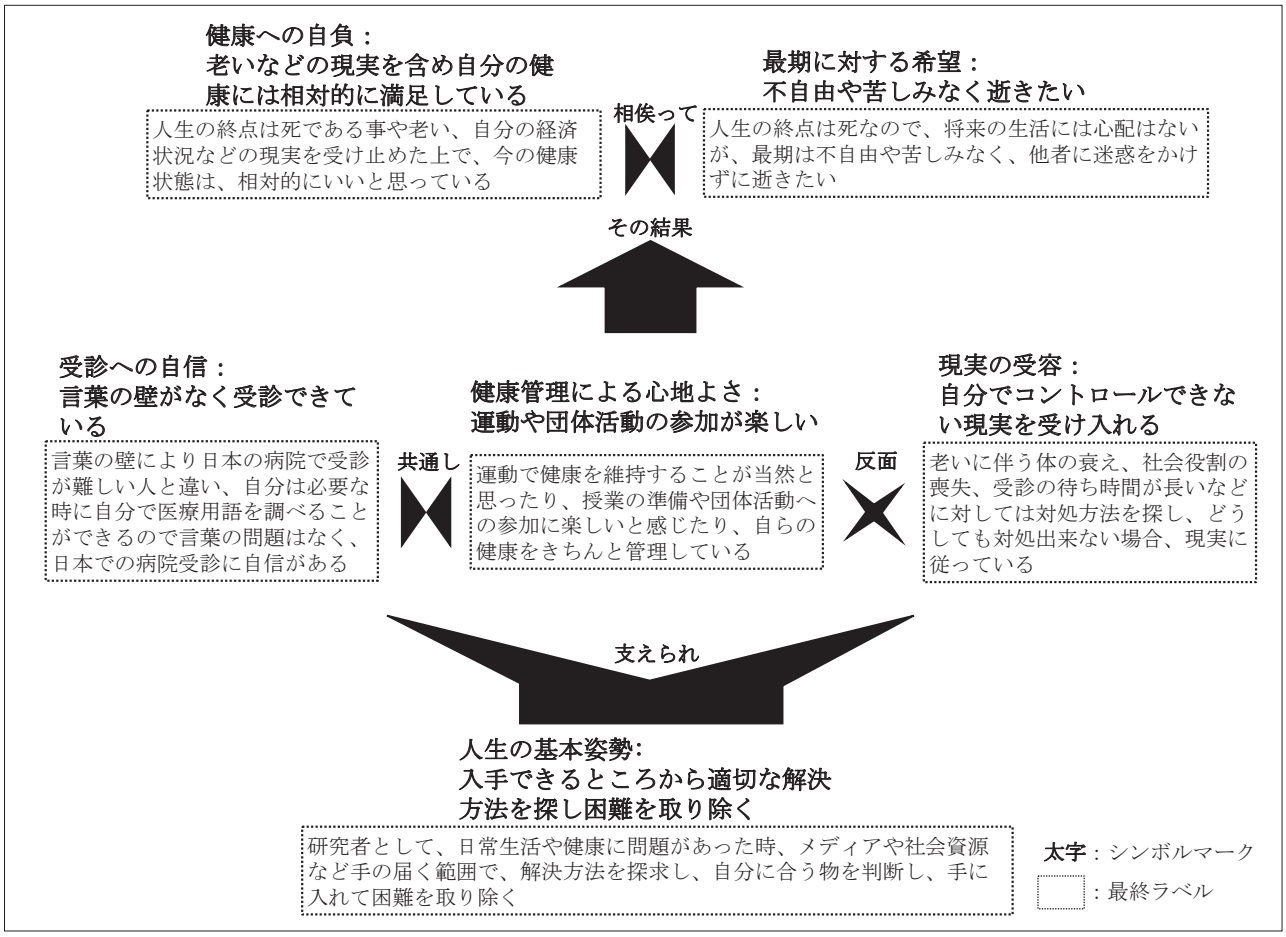

図1　A氏の健康管理の空間配置図

性を以下のように叙述化した。[　]はシンボルマークにおける［健康管理に関する要因］、「　」はシンボルマークにおける「その方法」を示している。

A 氏は「言葉の壁がなく受診できている」という［受診への自信］と「運動や団体活動の参加が楽しい」という［健康管理による心地よさ］を共通して感じている。一方、「自分でコントロールできない現実を素直に受け入れる」という［現実の受容］をしている。

そして、これらの健康管理の結果、「老いなどの現実を含め自分の健康には相対的に満足している」という［健康への自負］と「不自由や苦しみなく逝きたい」という［最期に対する希望］を持っている。

これらの健康管理は「入手できるところから適切な解決方法を探し、困難を取り除く」という［人生の基本姿勢］に支えられている。

考　察

A 氏の健康管理に関する語りの分析から得られた 6 つのシンボルマークについて考察する。

A 氏は必要時にわからない言葉を調べることで「言葉の壁がなく受診できている」という［受診への自信］を持っていた。世界保健機関はヘルスリテラシーを自身と周囲の人々の健康と幸福を促進し維持するために、情報やサービスへアクセスし、それを理解し利用するための個人的な知識とコンピテンシーと定義している[10]。これは健康に関する情報の理解や医療従事者に自分の心配を伝えるなどのヘルスケアに関するスキルに影響を及ぼす[11]。A 氏のように受診時に医療用語を調べるといった言葉の不安に対する事前の対応策を講じることは、在日中国人高齢者のヘルスリテラシーの発揮を促すと考えられる。

A 氏は太極拳やラジオ体操をきっかけにした地域の人との関わりなど「運動や団体活動の参加が楽しい」という［健康管理による心地よさ］を感じていた。移住者は文化や言葉の違いで移住した社会において、つながりを持つことに困難を感じる[5]。一方で、高齢者は社会参加機会の増加によって、地域における社会的ネットワークやサポートが充実し、社会的孤独やメンタルヘルスの改善、より良い身体的活動に繋がる[12]ことが報告されている。つまり、太極拳など中国文化にある健康管理方法は移住した社会における社会的つながりを促し、社会的サポートの充実とさらなる健康増進が期待できると言える。

A 氏は積極的な健康管理を行う一方で、歯の喪失や定年による引退などの「自分でコントロールできない現実を素直に受け入れる」という［現実の受容］をしていた。高齢期における生涯発達の課題には身体的健康の危機と引退の危機が含まれている[13]。守屋[14]は、高齢者は身体的機能の低下ならびに社会の役割などの喪失に直面せざるを得ないが、これらの事実を受け止めることは高齢期の自我発達につながると述べている。つまり、老いに伴う自分でコントロールできない［現実の受容］は、高齢者の心理社会的健康管理において重要であると考える。

これらの健康管理の結果として、A 氏は「老いなどの現実を含め自分の健康については相対的に満足している」という［健康への自負］と同時に、「不自由や苦しみなく逝きたい」という［最期に対する希望］を持っていた。そして、A 氏が実施した健康管理は「入手できるところから適切な解決方法を探し、困難を取り除く」という［人生の基本姿勢］に支えられていた。地域で暮らす高齢者は“自分の理想とする逝き方”、“いずれ訪れる死への準

備”に取り組んでおり、理想の最期を実現するために生前から死への準備を行うという特徴がある[15]。また、遠藤ら[16]は日常的に生じる困難や問題の解決策を見つけることができるという対処可能感は、直接男性高齢者の健康行動につながる促進要因であると報告している。A氏が実施している健康管理は健康への自負を生み出すとともに、最期の迎え方の希望につながっており、A氏の研究者としての経験が健康管理の行動を支えていた。医療従事者が在日外国人高齢者に対する健康促進策を検討する際、彼/彼女らの健康管理方法を理解することが重要であり、そのためには、彼/彼女らがそれまでの経験を通して構築してきた人生に対する姿勢を理解することの必要性が示唆された。

研究の限界

本研究は事例研究であるため、結果における個人的な傾向が強く、結果の一般化には限界がある。一方で、長期在日中国人高齢者であるA氏が日常生活の中で実施した健康管理およびそれに関する考えを深く探究できたことは、今後の在日中国人高齢者の健康促進の方策を検討するための一助となると考える。今後、在日中国人高齢者を対象とした研究成果を蓄積する必要がある。

結　論

本研究は地域で自立した生活を送る長期在日中国人高齢者A氏が行っている健康管理およびそれに関連する考えを明らかにした。A氏は［人生の基本姿勢］に支えられた［受診への自信］を持ち、［健康管理による心地よさ］を感じている。一方［現実の受容］をしており、これらの健康管理の行動の結果、［健康への自負］と［最期に対する希望］を持っていた。

謝　辞

本研究にご理解とご協力をいただいたA氏に謹んで御礼申し上げます。本研究は日中笹川医学奨学金より助成を受けて実施し、研究者の千葉大学大学院修士論文の一部を加筆・修正したものであり、The 9th Hong Kong International Nursing Forum cum 1st Greater Bay Area Nursing Conference にてポスター発表した。本研究における利益相反は存在しない。

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