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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）
第４４期（学位取得コース）

中　間　報　告　書

2022 年 4 月～2023 年 3 月

公益財団法人　日中医学協会

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日中笹川医学奨学金制度(学位取得コース)中間評価書
課程博士：指導教官用

第 43 期　　研究者番号：G4301　　作成日　：2023 年 3 月 10 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
学生本人が行った研究の概要	（背景と目的）多能性幹細胞はあらゆる細胞へ分化する能力を有し、再生医療や疾患病態の解明、薬剤スクリーニングなど様々な使用用途がある。多能性幹細胞から特定の細胞への分化には一般的に多くの試薬の添加と厳密な分化スケジュールの管理が必要となる。試薬の添加による分化誘導法はこれまで数多く報告されているが、細胞を培養するプレート、基質と分化との関連については報告が少なく、その大部分が基質の硬さとの関連にとどまる。基質には硬さ、新疎水性、濡れ性、粗さ、表面電位など様々な物性があり、複雑な微小環境を形成している。当研究室では基質表面に様々な荷電を付加した荷電ハイドロゲルを作成し、多能性幹細胞の中胚葉、軟骨細胞への分化について研究を進めてきた。 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 ・human 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 ・基質荷電を用いた新たな分化プロトコールを確立する。 ・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。	
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 細胞は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。 【改善すべき点】 特になし 【今後の展望】 樹立した細胞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、従来法で作成したドパミン作動性細胞との比較を行う。順調で有れば、大学院４年目は論文がまとめ、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	

日中笹川医学奨学金制度(学位取得コース)中間報告書
研究者用

第43期　　研究者番号：G4301　　作成日：2023年3月10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)				
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	☑	

1．研究概要(1)

背景と目的)多能性幹細胞はあらゆる細胞へ分化する能力を有し、再生医療や疾患病態の解明、薬剤スクリーニングなど様々な使用用途がある。多能性幹細胞から特定の細胞への分化には一般的に多くの試薬の添加と厳密な分化スケジュールの管理が必要となる。試薬の添加による分化誘導法はこれまで数多く報告されているが、細胞を培養するプレート、基質と分化との関連については報告が少なく、その大部分が基質の硬さとの関連にとどまる。基質には硬さ、新疎水性、濡れ性、粗さ、表面電位など様々な物性があり、複雑な微小環境を形成している。当研究室では基質表面に様々な荷電を付加した荷電ハイドロゲルを作成し、多能性幹細胞の中胚葉、軟骨細胞への分化について研究を進めてきた。

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。

1)目的(Goal)

荷電ハイドロゲルを用いたhuman iPS細胞のドパミン作動性細胞分化の解析

2)戦略(Approach)

多分化能をもつiPS細胞は荷電ハイドルゲルで培養して、より効率誘導分化することが出来る、さらに安定したドパミン産生する細胞株の樹立し、アルツハイマーを根治することが期待出来る。

3)材料と方法(Materials and methods)

・human 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
・基質荷電を用いた新たな分化プロトコールを確立する。
・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。

4)実験結果(Results)

①iPS細胞は荷電ハイドルゲル培養について：我々はiPS細胞を用いて、荷電ハイドルゲル培養は成功した。より効率かつ安定した神経に分化することが判明した。②AADC Konckdown細胞株の樹立：AADC Knockdown細胞株を樹立した、WBでAADCの発現量は低下したことが確認した。③荷電ハイドルゲルを用いて、AADC KnockdownしたiPS細胞を培養して、よりドパミン産生を促進することが確認した。ドパミン産生量はWBで増加したことが確認出来た。

5)考察(Discussion)

現在はiPS細胞を用いて、荷電ハイドルゲルで培養して、より効率かつ安定な神経分化することは判明し、さらにAADC KnockdownしたiPS細胞株は荷電ゲルで分化誘導する際にドパミン産生量は安定的誘導することが出来た、さらに荷電ハイドルゲルで培養は通常培養よりドパミン産生量の増加がWBで確認した。今後はHPLC法でドパミンを確認し、in vivoでアルツハイマーモデルを作成し、荷電ハイドルゲル培養したiPS AADC Knockdown細胞株を移植し、治療を行う予定です。

6)参考文献(References)

[1]Doi D., Samata B., Katsukawa M., et.al. Isolation of Human Induced Pluripotent Stem Cell-Derived Dopaminergic Progenitors by Cell Sorting for Successful Transplantation[D]. Stem Cell Reports, 2014, 02(03), 337-350.
[2]Tushar Kamath, Abdulraouf Abdulraouf, S. J. Burris, Jonah Langlieb, Vahid Gazestani, Naeem M. Nadaf , Karol Balderrama, Charles Vanderburg and Evan Z. Macosko. Single-cell genomic profiling of human dopamine neurons identifies a population that selectively degenerates in Parkinson's disease[D]. Nature Nueroscience, 2022(25), 588-595.
[3]Gabriel E. Hoffman, Brigham J. Hartley, Erin Flaherty, Ian Ladran, Peter Gochman,
[4]Douglas M. Ruderfer, Eli A. Stahl, Judith Rapoport, Pamela Sklar & Kristen J. Brennand. Transcriptional signatures of schizophrenia in hiPSC-derived NPCs and neurons are concordant with post-mortem adult brains[D]. NATURE COMMUNICATIONS. 8: 2225 (2017)
[5]Sameehan Mahajani, Anupam Raina, Claudia Fokken, Sebastian Kügler and Mathias Bähr. Homogenous generation of dopaminergic neurons from multiple hiPSC lines by transient expression of transcription factors. Cell Death & Disease 10: 898 (2019)

1．研究概要(2)多能性幹細胞はあらゆる細胞へ分化する能力を有し、再生医療や疾患病態の解明、薬剤スクリーニングなど様々な使用用途がある。多能性幹細胞から特定の細胞への分化には一般的に多くの試薬の添加と厳密な分化スケジュールの管理が必要となる。試薬の添加による分化誘導法はこれまで数多く報告されているが、細胞を培養するプレート、基質と分化との関連については報告が少なく、その大部分が基質の硬さとの関連にとどまる。基質には硬さ、新疎水性、濡れ性、粗さ、表面電位など様々な物性があり、複雑な微小環境を形成している。当研究室では基質表面に様々な荷電を付加した荷電ハイドロゲルを作成し、多能性幹細胞の中胚葉、軟骨細胞への分化について研究を進めてきた。
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。

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		
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 3 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 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					
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			
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	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	
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）　谷川 聖

日中笹川医学奨学金制度(学位取得コース)中間評価書
課程博士：指導教官用

第 43 期　　研究者番号：G4302　　作成日　：　2023 年 3 月 6 日

氏　名	趙 雪	ZHAO XUE	性別	M	生年月日　1985/02/09
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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(前立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	優　良　可　不可 学業成績係数=4.0	取得単位数 21／30
学生本人が行った研究の概要	この一年間の研究活動では、アミノ酸トランスポーターに関する 2 つのレビュー論文と前立腺癌に関する 1 つの臨床論文をいずれも英文雑誌に発表した。英文単行書の 1 章も執筆した。前立腺癌とアミノ酸トランスポーターの課題に加えて、膀胱癌とアミノ酸トランスポーターに関する課題についても博士論文に関連する重要な課題として取り上げた。いずれも現在、精力的に進めている。また、当学生は 10 年以上の臨床経験があり、泌尿器科学教室で行っている臨床研究にも積極的に参加しており、前立腺癌や人工知能に関する領域についても着手している。	
総合評価	【良かった点】 当学生の研究に臨む姿勢は申し分なく、前立腺癌とアミノ酸トランスポーターの研究のみならず、膀胱癌と人工知能関連の臨床研究や各種研修への協力などを実践している。臨床的な問題意識と論理的なアプローチの両者においてバランスよく、 研究に邁進できている。今後の大きな研究成果とさらなる飛躍が期待される。 【改善すべき点】 ① 計画立案・準備能力の向上: 研究は複雑で難しく想定外のことが多発するので、独立して行う研究企画能力をさらに伸ばしていくことを期待している。 ② 個人の限界を上げる作業: ポジティブ思考の行動パターンやストレッチ目標に挑戦する姿勢をさらに高め、さらに高度な能力を獲得することを期待している。 【今後の展望】 今後は課題計画に沿って基礎実験を進め、客観的な結果を得たうえで博士論文を完成させる予定である。それと同時に、できる限りの臨床研究を行い、英文論文として発表していく予定である。これらを通じて、泌尿器科学の発展に貢献してくれることを期待している。	
学位取得見込	まじめに、着実に研究計画を実行し、研究室内の他の研究業務にも積極的に参加することにより、本人の優れた学修能力と責任感ならびに実行力を実証した。学修生活において課題に直面しても、すぐれた個人の能力と周囲との協調性を重んじる人柄によって克服している。既に期待を遙かに上回る成果を挙げており、博士号取得は全く問題無く順調に進んでいると評価する。	
評価者（指導教官名）	市川 智彦	

日中笹川医学奨学金制度(学位取得コース)中間報告書
研究者用

第43期　　研究者番号：G4302　　作成日：2023年3月4日

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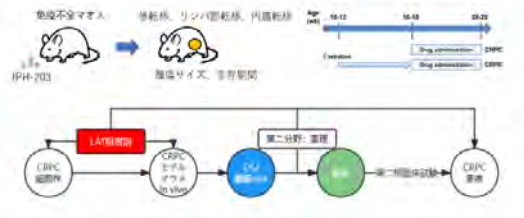
研究先（指導教官）	千葉大学大学院 医学研究院泌尿器科学（市川 智彦　教授）				
研究テーマ	アミノ酸トランスポーターを介した前立腺癌分子機構の解明（前列腺癌とアミノ酸トランスポーター） Elucidation of the molecular mechanism of prostate cancer via amino acid transporter (prostate cancer and amino acid transporter)				
専攻種別	論文博士	□	課程博士	☑	

1．研究概要（1）

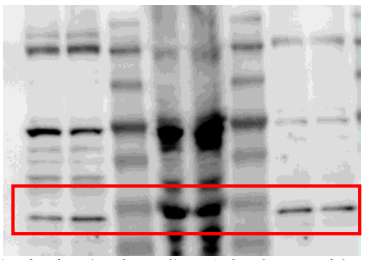
1）目的（Goal）
目的：①アミノ酸トランスポーター（LAT）阻害剤の去勢抵抗性前立腺癌（CRPC）に対する作用効果の解明（In vivo）。②LAT1を含めたトランスポーターを介したCRPC治療モデルの構築と、実臨床への応用を目指す。
研究背景：社会の高齢化が進むにつれて、前立腺癌の発病率は年々増加している。前立腺癌の治療において、無視できないのは、前立腺癌が最終的に去勢抵抗型前立腺癌（CRPC）に転換することである。我々は、前立腺癌がCRPCに移行するメカニズムとして、アンドロゲン受容体（AR）に制御されるトランスポーターとしてLAT1（LAT1-4F2hcヘテロダイマー型トランスポーター）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。

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 ~ 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)
LAT1の高発現は3種類の腫瘍細胞から検出されている。
赤枠内がLAT1です。



期待結果:LAT1ノックアウト後のrtPCR検証、
免疫不全マウス移植腫瘍のサイズ比較、
免疫不全マウスモデルのOS生存曲線比較分析です。

5)考察(Discussion)
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。

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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					
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さい

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

学会名 Conference	なし				
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			
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	
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)　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

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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

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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.

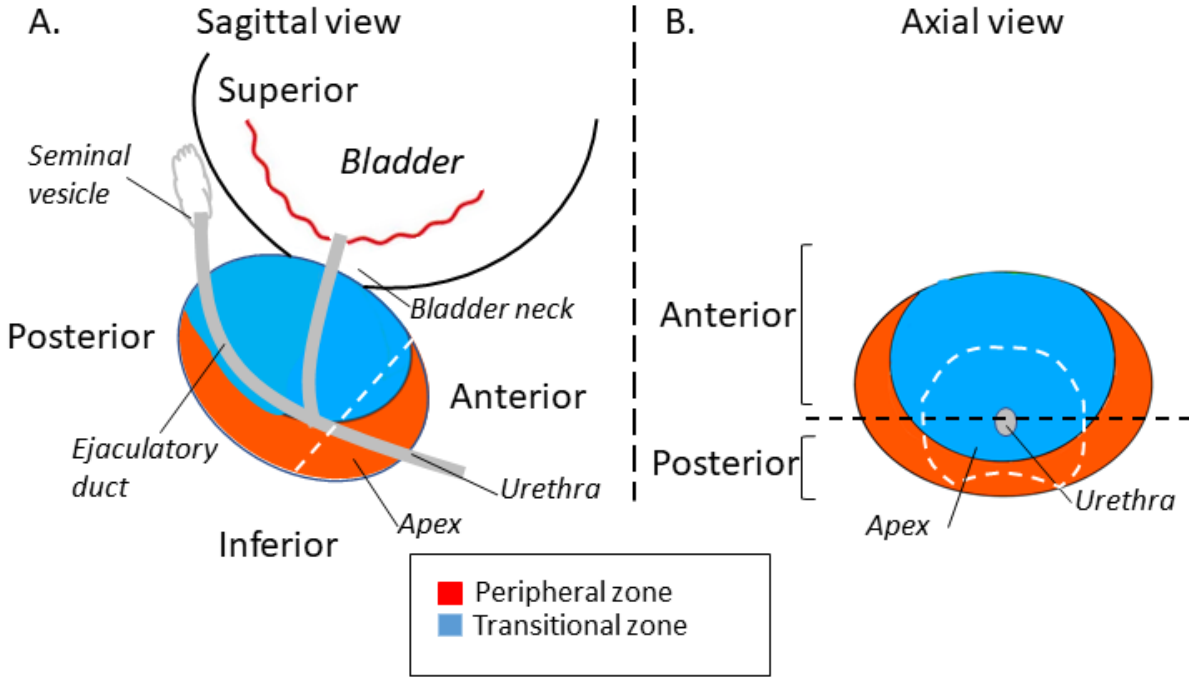

Figure 1. Schematic diagram of an anatomical division of the prostate. The location of the Anterior/Posterior and Peripheral/Transitional Zones are described. (**A**) Sagittal view. (**B**) Axial view.

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

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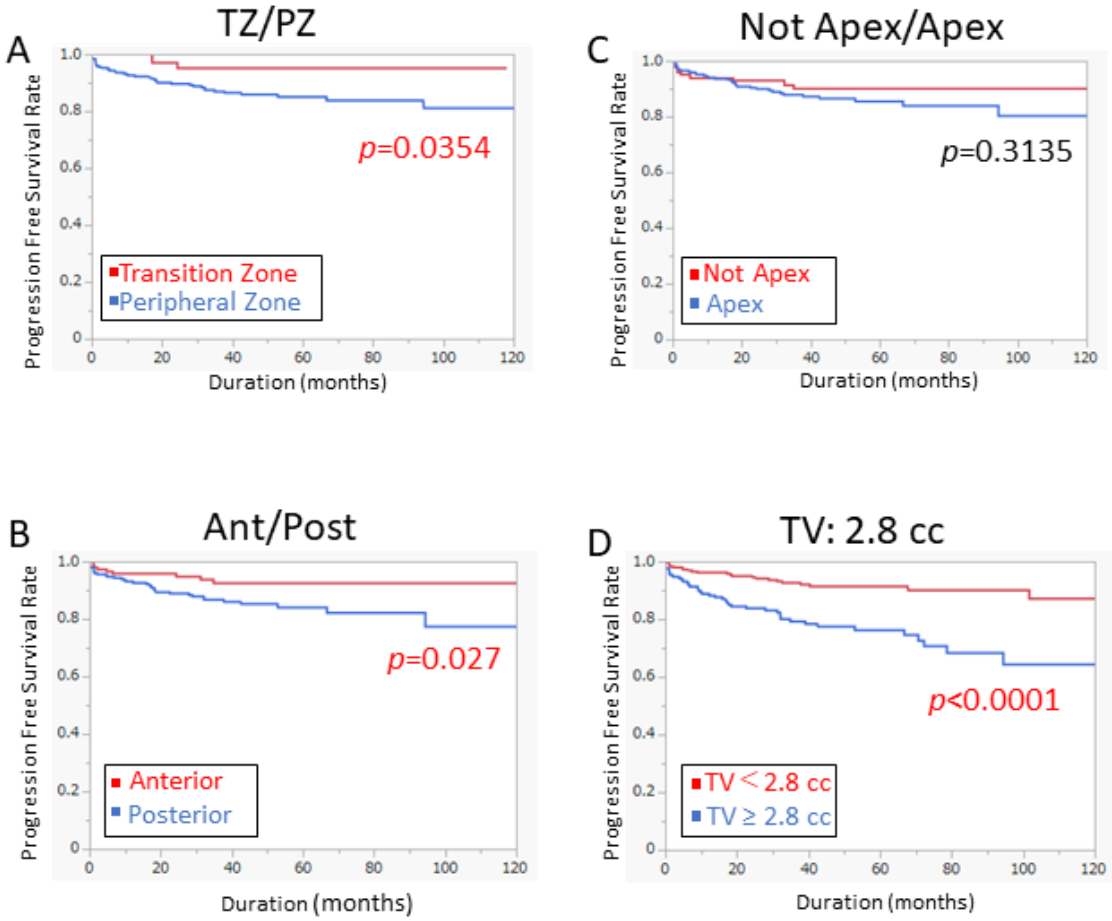

Figure 2. Prognostic significance of tumor location and tumor volume. (**A**) Patients with tumor in the PZ had significantly worse PFS than those in the TZ ($p = 0.0354$). (**B**) Patients with tumor in the posterior region had significantly worse PFS than those in the anterior region ($p = 0.027$). (**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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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 our 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 shown in Table 3 and only these risk factors predicted BCR on multivariable analysis (HR 3.16 [95% CI: 1.52–6.56] $p = 0.002$).

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	1.00–2.73	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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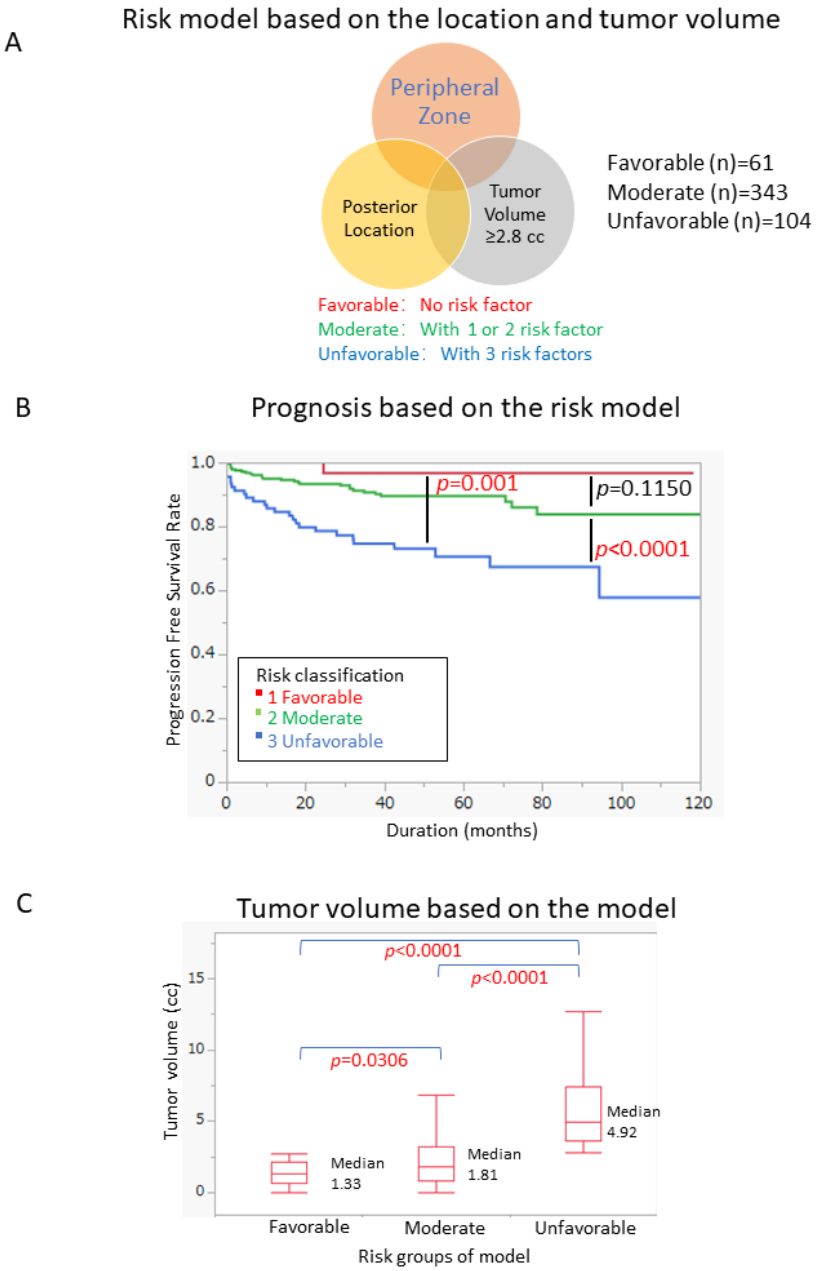

Figure 3. Prognostic model based on the location and tumor volume (**A**) Venn diagram of risk model based on the location and tumor volume. (**B**) Risk classification significantly differentiated the PFS between the Favorable and Unfavorable group ($p < 0.0001$) and the Moderate and Unfavorable group ($p < 0.0001$). (**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 $p = 0.0873$).

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 ≥ 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 ≥ 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 $p = 0.0873$).

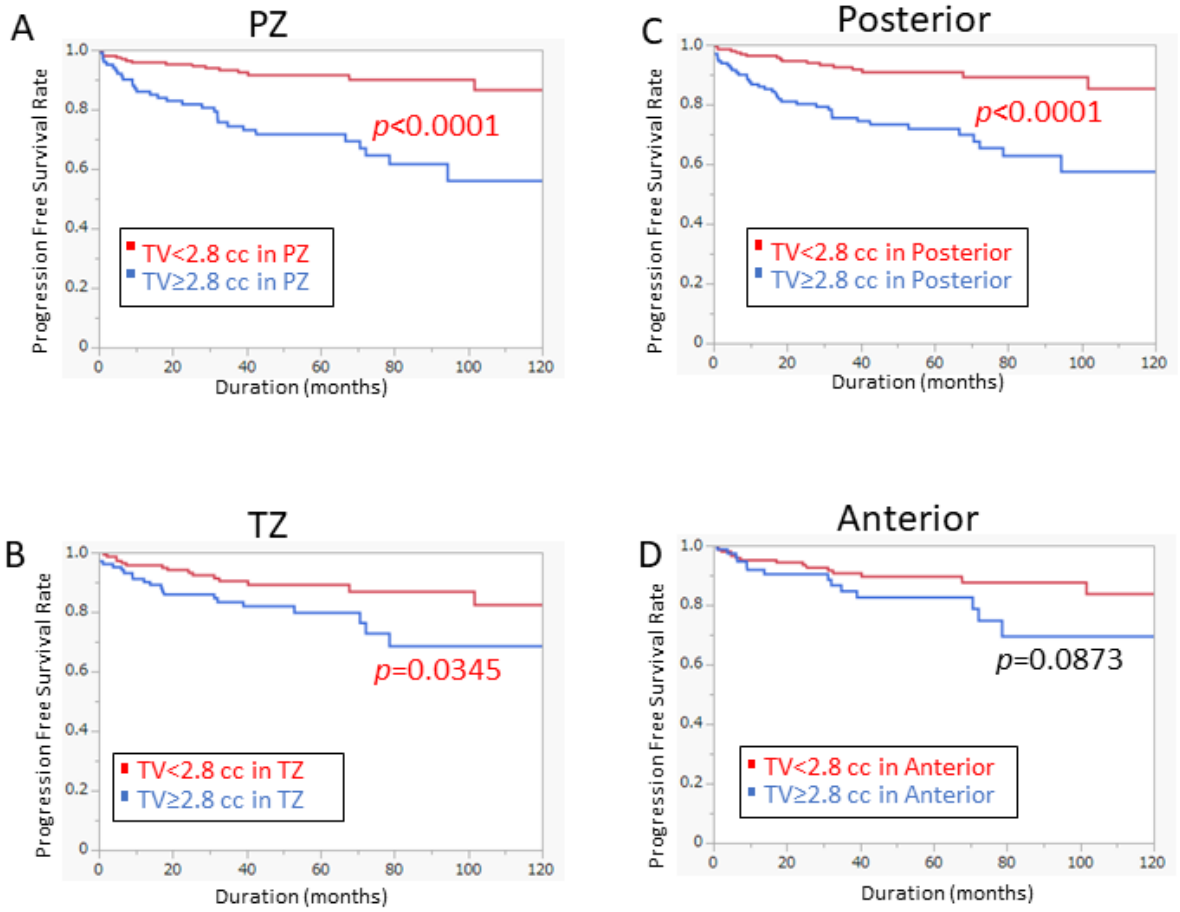

Figure 4. Prognostic significance of Tumor volume 2.8 cc based on the location. (**A**) Patients with tumor volume ≥ 2.8 cc had significantly worse PFS in the PZ ($p < 0.0001$). (**B**) Patients with tumor volume ≥ 2.8 cc had significantly worse PFS in the TZ ($p = 0.0345$). (**C**) Patients with tumor 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 ($p < 0.0001$).

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

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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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Full paper

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

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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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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

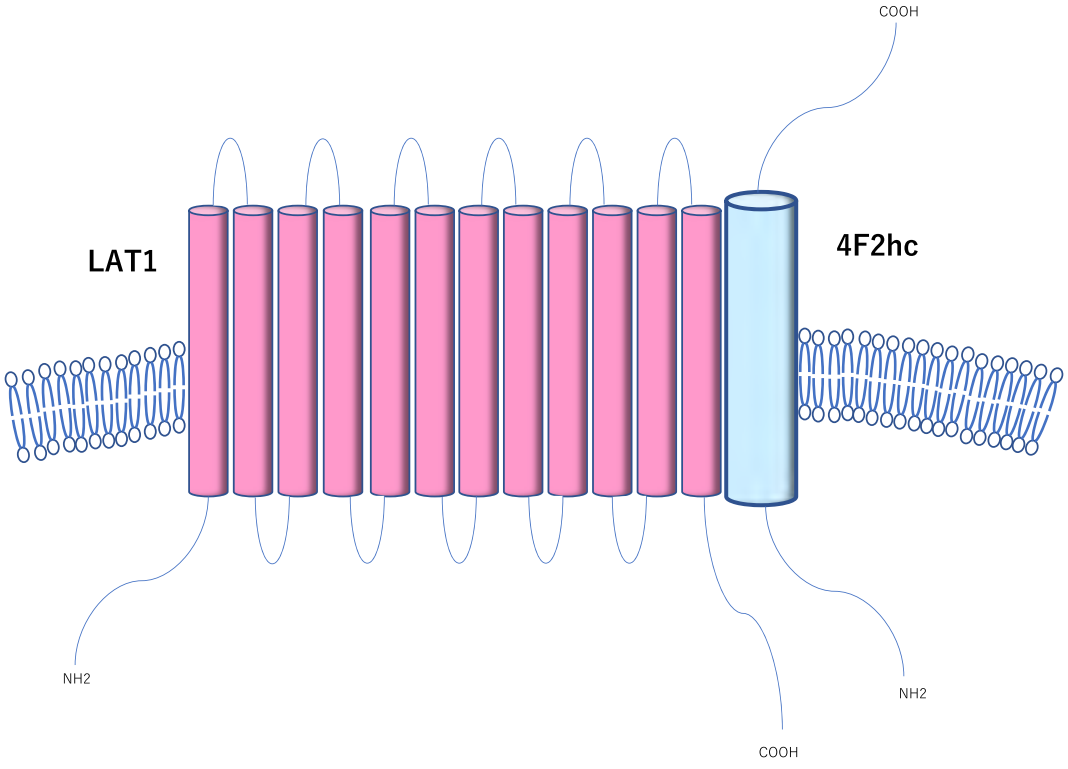

Fig. 1. Scheme shows the representation of LAT1/4F2hc complex. The structures of the LAT1 and 4F2hc heterodimers on the plasma membrane are shown in simplified form: LAT1 is a 12-fold transmembrane amino acid transporter that serves as the light chain of the dimer; 4F2hc is a single-fold transmembrane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

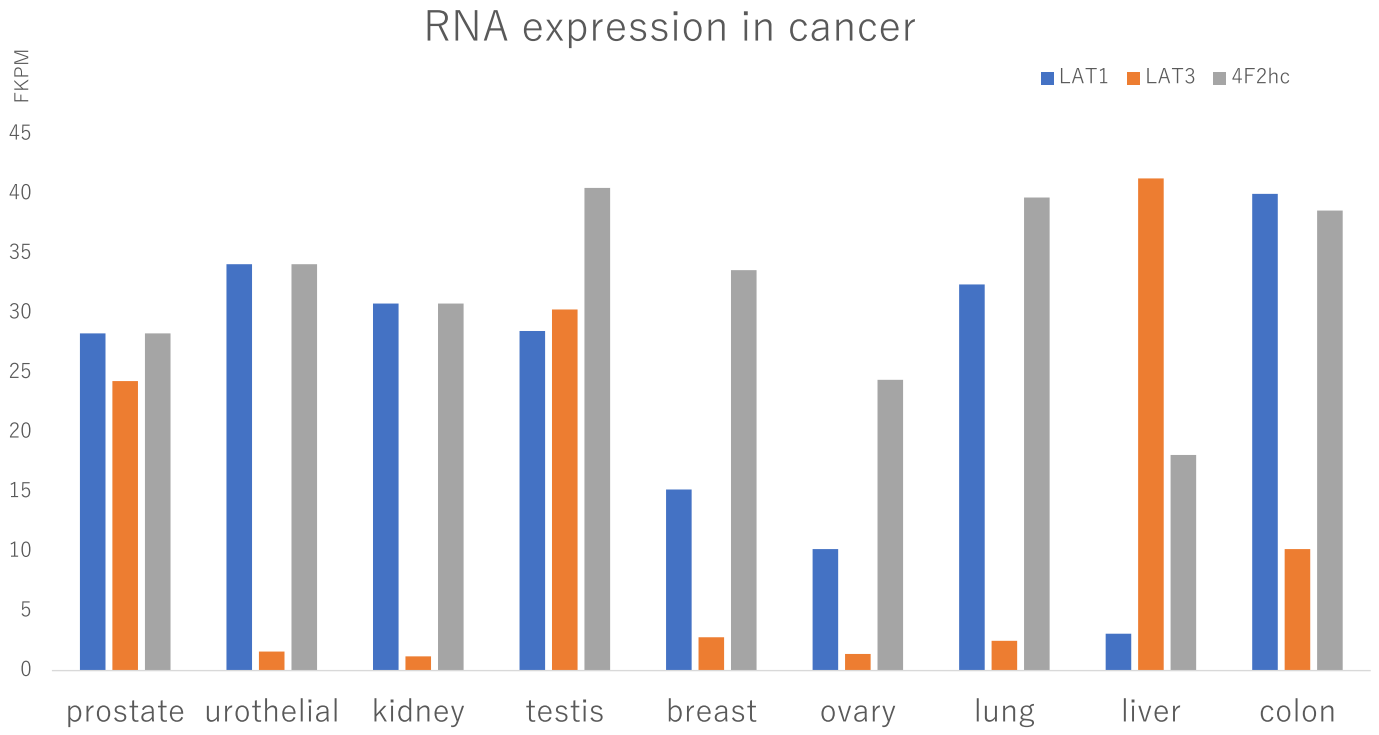

Fig. 2. The expression of LAT1, LAT3, and 4F2hc in tumor cells is shown, and LAT1 expression is more upregulated in many tumors than LAT3, which is the reason why LAT1 may be the tumor cell type amino acids transporter. However, in prostate and testicular cancers, LAT3 is expressed to the same extent. 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]

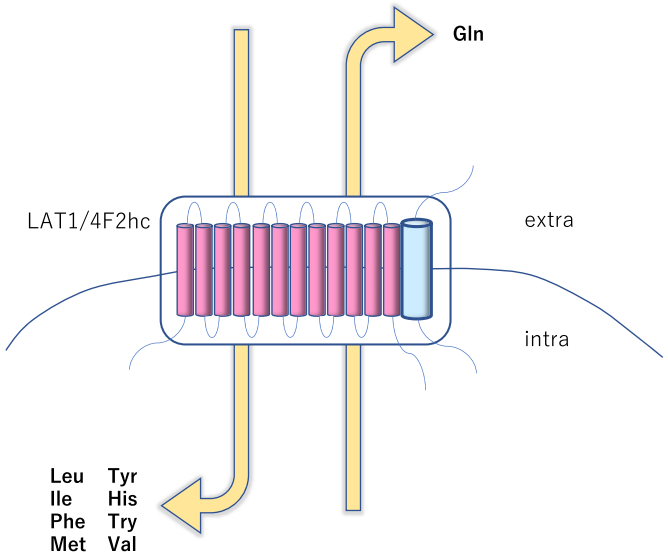

Fig. 3. Scheme shows the substances of LAT1/4F2hc. LAT1 is sodium-independent and transports a single molecule of glutamine out of the cell, and at the same time takes up large side-chain neutral amino acids such as leucine, histidine, methionine, isoleucine, valine, phenylalanine, tyrosine, and tryptophan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

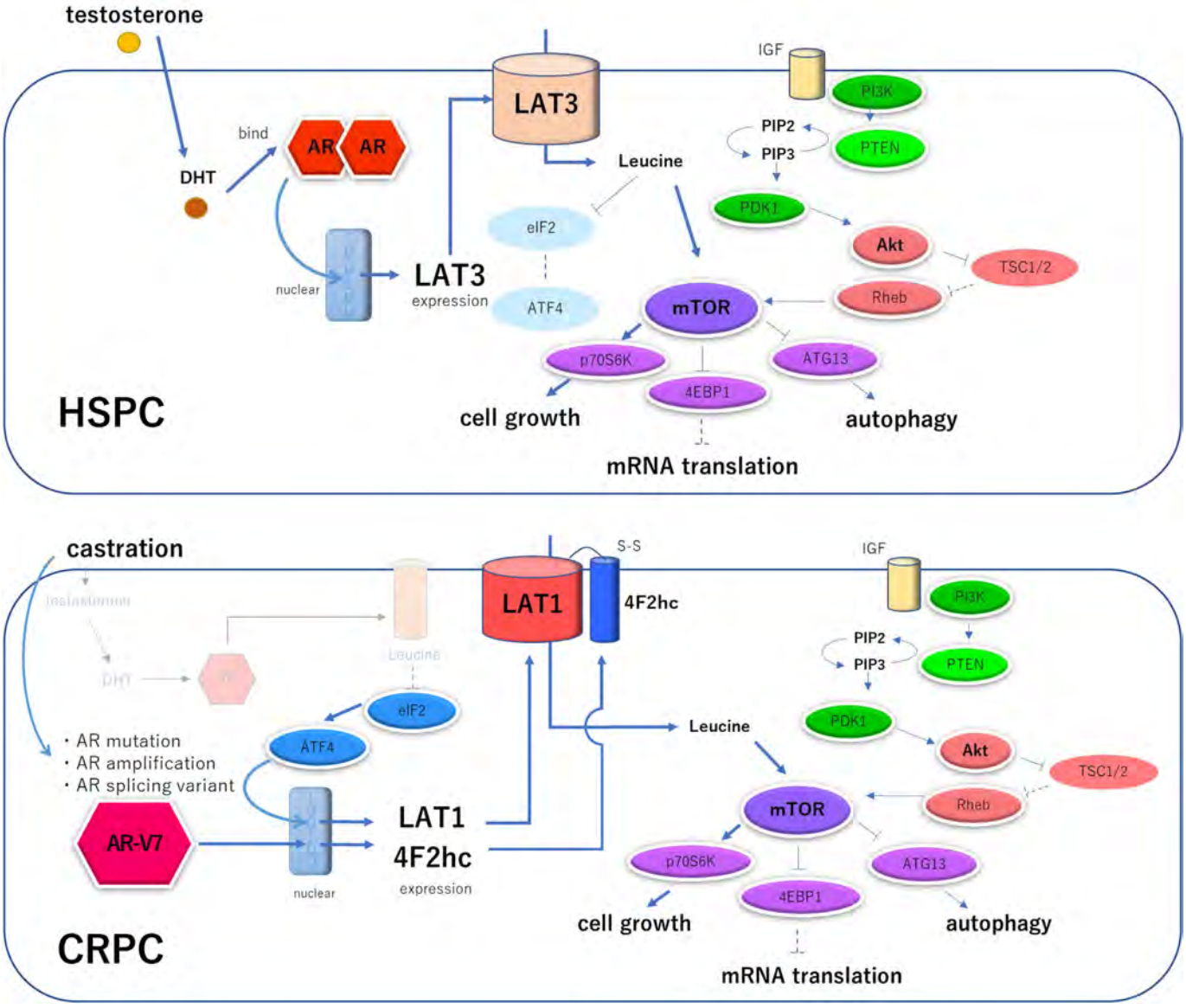

Fig. 4. Scheme shows the association of LATs and prostate cancer. The association of hormone-sensitive prostate cancer (HSPC) and castration-resistant prostate cancer (CRPC) with LAT is shown. In untreated HSPC, testosterone is metabolized by 5-alpha reductase to dihydrotestosterone (DHT), which binds 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.

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Review

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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

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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).

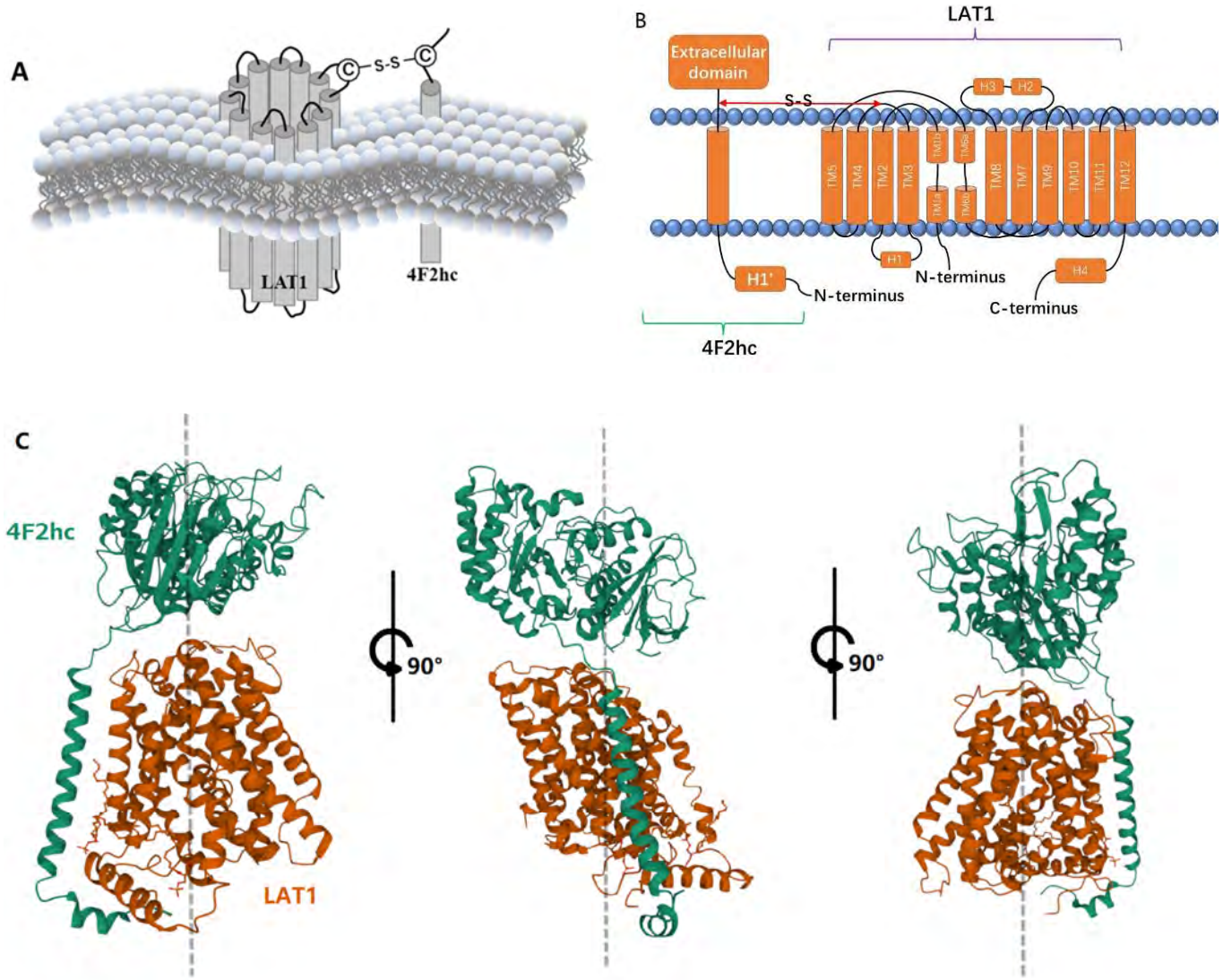

Figure 1. Structure of LAT1-4F2hc Complex: (**A**) Hypothetical model of the complex of LAT1 and 4F2hc; (**B**) LAT1 has 12 transmembrane units, while 4F2hc has only one. The two are covalently connected by disulfide bonds; (**C**) FIG1 (**C**) Images created using Mol*, the PDB ID: 6IRS, Structure of 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).

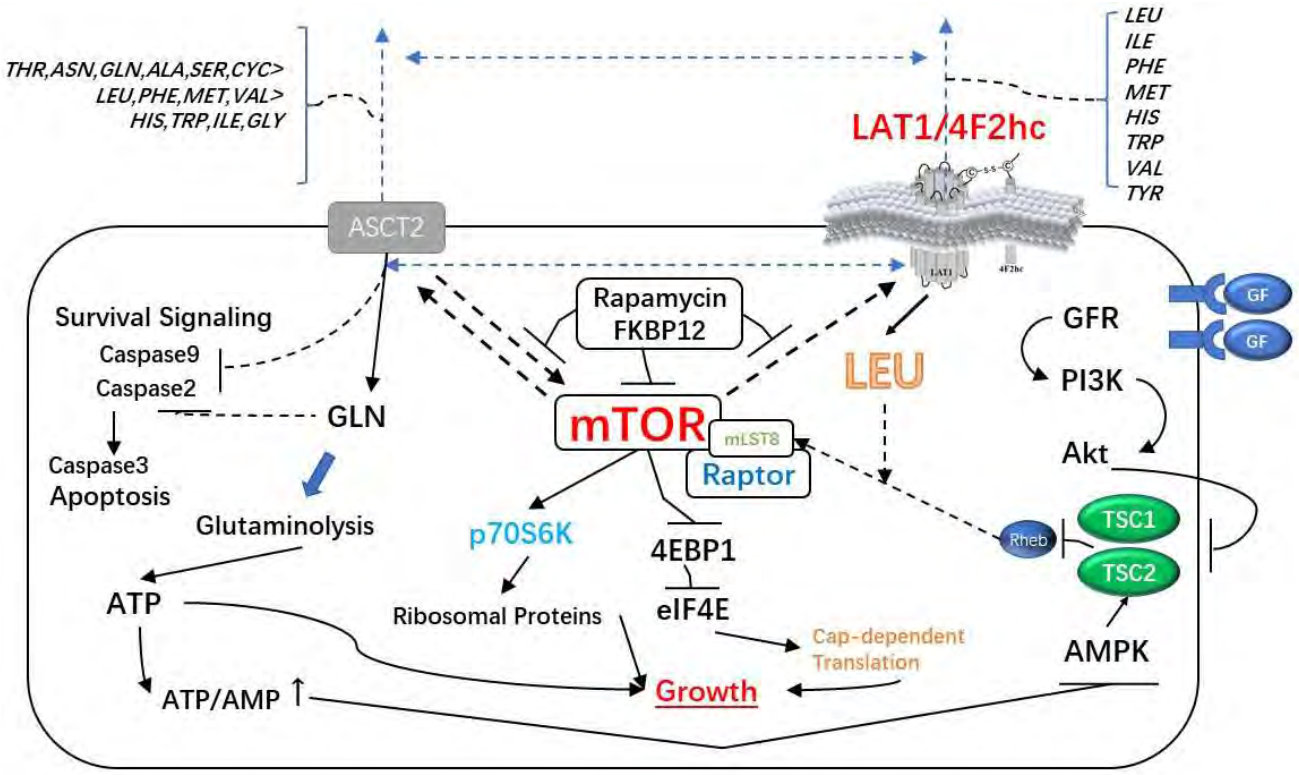

Figure 2. The Major Signaling Pathways Affected by LAT1-4F2hc Complex: The LAT1-4F2HC complex not only enhances mTORC1 activity but also enhances MYC and EZH2 signaling pathways. Moreover, it can affect the proliferation of cancer cells through AKT, MAPK and cell cycle-related P21 and P27 signaling pathways.

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).

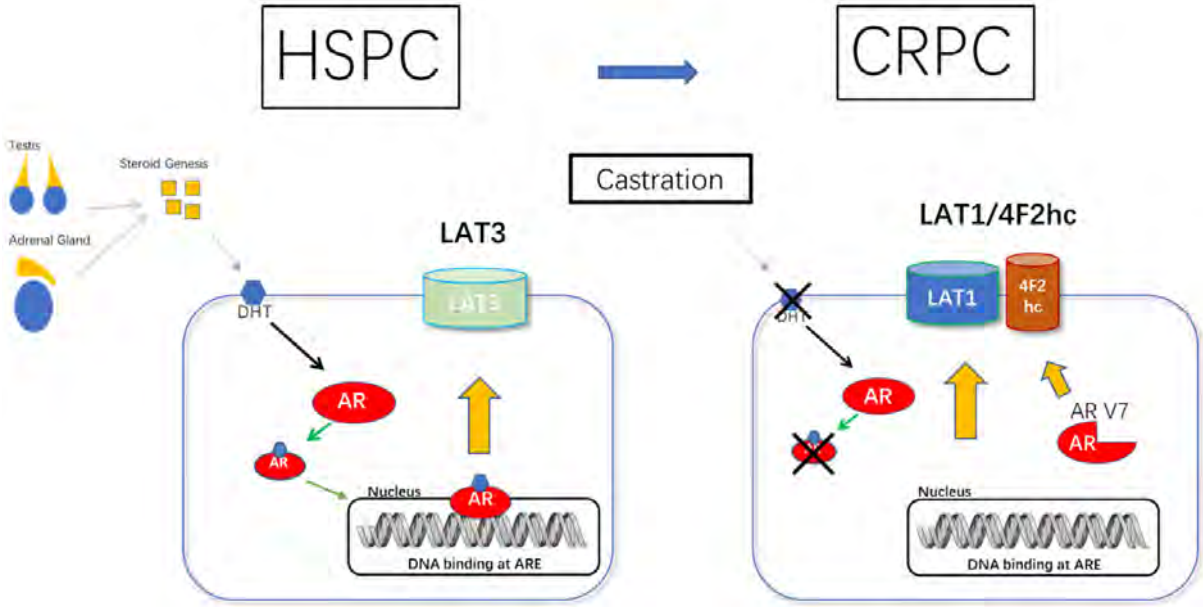

Figure 3. LAT Expression is Coordinately Regulated During Prostate Cancer Progression: Proposed model of LAT1-4F2hc/LAT3 in HSPC to CRPC. As HSPC progresses to CRPC, AR acts in reverse to cause low expression of LAT3 and high expression of LAT1.

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).

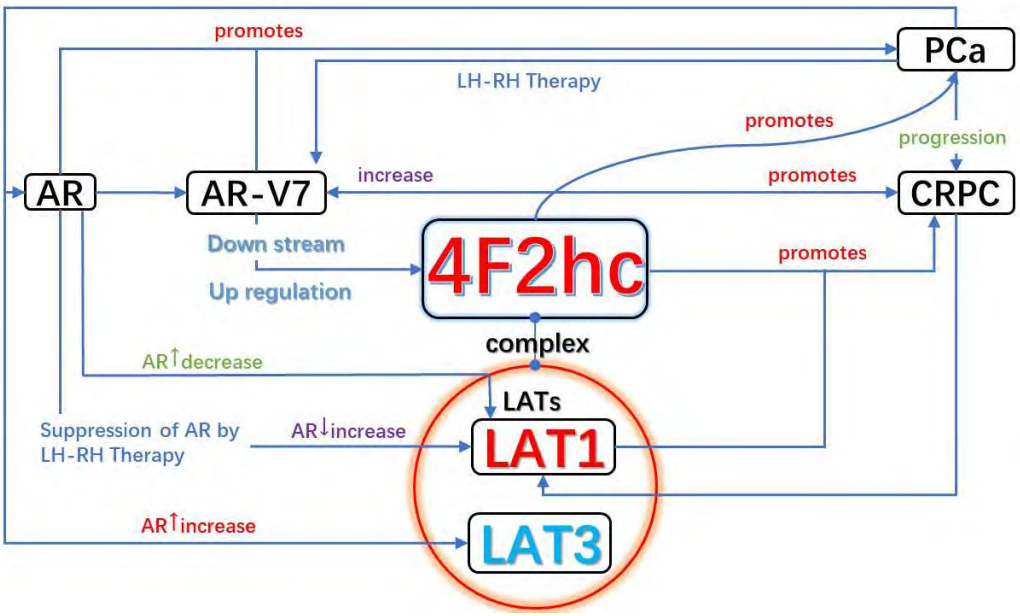

Figure 4. Relationship of LAT1-4F2hc and PCa & CRPC: The relationship between LATx-4F2hc and AR(AR-V7) and different stages of prostate cancer. Reduced androgen receptor signaling and variation of androgen receptors may result in decreased LAT3 expression and higher LAT1 expression.

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.

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Chapter 3.

Serum Testosterone Level Determines 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 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

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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

Background

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. Supraphysiologic 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.
   First, 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 came to the conclusion 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 reexposed 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).

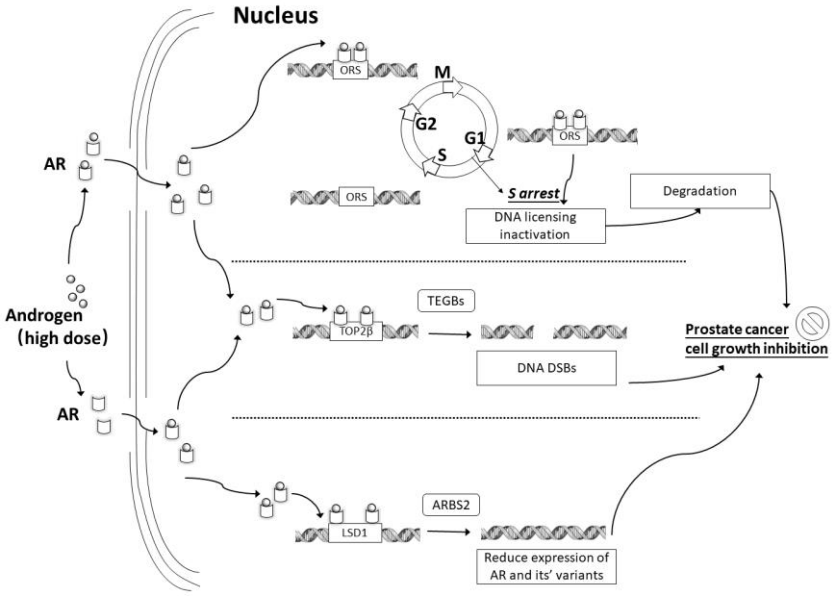

Figure 1. Mechanism of high dose androgen inhibiting the growth of prostate cancer cells.

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.

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 $\geqslant$ 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]

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].

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.

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).

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

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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成績状況	優　(良)　可　不可	取得単位数 取得単位数／取得すべき単位総数
学生本人が行った研究の概要	免疫学的機序により進展する糸球体腎炎のうち、アジアで最も多い IgA 腎症をテーマに、 IgA 腎症の進展機序の解明を目的に、マウスモデルを用いて実験を進めている。 自然発症 IgA 腎症のモデルとなる ddY マウスのうち、本邦の武曾らにより 1996 年に確立された high IgA ddY (HIGA)マウスを用いて、全身の炎症状態を増悪するグラム陰性桿菌の菌体成分で炎症性サイトカインの放出を促進する Lipopolysaccharides (LPS)を複数回投与して、IgA 腎症の進展について、病理学的に解析している。現在、10 週令の HIGA マウスに LPS 60μg もしくは 80 μg を 1 週間ごとに腹腔内投与して、尿所見、腎機能、および IgA 腎症の組織学的進展を確認している。投与開始後 1 週目には蛋白尿がみられ、30 週目には蛋白尿の増悪と血尿の出現を認めている。これらの尿所見の増悪と腎糸球体の病理学的所見との関連を検討している。今後は尿所見の増悪に関連する糸球体所見、免疫学的特徴、免疫グロブリンの沈着や補体の関与、サイトカインやケモカインの変化、単離糸球体内での蛋白群の変化や mRNA を用いた糸球体内細胞群の活性化や形質転換、免疫学的活性化経路の解析などを進める予定である。	
総合評価	【良かった点】 教室にきて 1 年間で、倫理講習、実験動物室講習を行い、動物実験のためのモデルを決定し、動物実験を開始している。その解析方法についても、尿や血液所見の解析方法、実験動物の屠殺や臓器摘出の方法などを取得し、病理解析方法も講習を受け、解析を進めている。実際に自分で行うことがてきていることは評価できる。また、論文を読み、仮説を立て、実証しようする姿勢には共感を持てる。 【改善すべき点】 論文からの情報を入れ、仮説を立てる作業には共感が持てるが、それをどのように自身の実験に活かしていくのかの具体的な方法論の構築がまだ出来ていない。自身が行ってきた実験の結果を丁寧に観察し、その実験結果からの仮説の検証、その結果を踏まえて、次なる展開の方向性を決めていく過程の成熟にはさらなる時間が必要のようである。自身が行った実験の検体を大切にし、詳細に観察して、それを基盤に実験を積み上げていくよう、指導している。	

	【今後の展望】 現在行っている HIGA マウスを用いた IgA 腎症マウスモデルを用いて、LPS による免疫活性化モデルでは尿の増悪が確認され、現在、腎糸球体の病理所見の増悪を確認している。尿所見の増悪に関連する病理所見の同定は、臨床の IgA 腎症の腎生検所見を診断する際に重要になる。さらに、尿所見の増悪に関連する免疫学的特徴、免疫グロブリンの沈着や補体の関与、サイトカインやケモカインの変化、単離糸球体内での蛋白群の変化や mRNA を用いた糸球体内細胞群の活性化や形質転換、免疫学的活性化経路の解析などを進める予定である。また、臨床で確認される一過性感染症による急性活動性病変の形成のための腹膜炎惹起モデル、コロナワクチン後のIgA腎症の増悪に関連するワクチン成分投与モデルなど、短時間で確実にHIGAマウスの IgA 腎症が増悪するモデルの新規開発も並行して進める予定である。マウスモデルでの in vivo の実験結果は、臨床でのヒト IgA 腎症の解析の糸口を示す大切な研究である。今後の展望として、HIGA マウスの IgA 腎症の進展経過と、その進展機序を解明し、臨床のヒト IgA 腎症の症例の腎生検検体で確認を行い、マウス実験から臨床への応用が可能な病理所見や、進展過程の免疫学的動態を明らかにするために研究を続ける。
学位取得見込	江傑先生は熱心に研究を継続しており、HIGA マウスを用いた IgA 腎症の実験モデルで、LPSを複数回腹腔内投与することで、すでにIgA腎症の増悪モデルを確立している。この解析に入っており、これらを丁寧に進めることで、新規結論を導き出すことが可能で、それらをまとめることで、学位取得は十分に見込めると考え、指導している。
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研究先(指導教官)	日本医科大学医学部解析人体病理学 (清水 章 教授)				
研究テーマ	腎疾患の進展機序の解明とその制御 The elucidation of the mechanism of the development of renal disease, and its control				
専攻種別	論文博士	☑	課程博士	☐	

1. 研究概要(1)

1) Aim:

The clarify the mechanisms for the progression of IgA nephropathy, using the experimental animal model of IgA nephropathy (HIGA mice) with progressive hematuria, albuminuria and renal dysfunction.

2) Approach:

Vascular endothelial cells form the inner layer of blood vessels where they have a key role in the development and maintenance of the functional circulatory system and provide paracrine support to surrounding non-vascular cells. And one critical piece of the defense network that comprises the innate immune system is the endothelium. The kidney is an organ rich in vascular structure, with a wide range of uniquely differentiated endothelial cells, which are susceptible to damage from circulating bacteria, toxins, cytokines and immune complexes. At the same time, it also has the ability to interact with circulating immune cells and a self-protection mechanism. The recurrence and aggravation of immune-related nephropathy caused by upper respiratory tract infections and acute COVID-19 infection have aroused many thoughts in clinical practice. It can be considered that innate immunity initiated by endothelial cell damage plays a very important role in these progresses, but it's mechanism remains unclear.

Understanding the difference in the human endothelium is emphasized by the role it plays in vascular homeostasis and innate immunity activation, making its modulation a key driver of the host response to infection. It has been postulated that cells of innate immunity develop tolerance in the setting of repeated TLR stimulation. While, these observations have been demonstrated in murine macrophages, humans have been shown to have both similar and divergent mechanisms of TLR modulation, suggesting that the mechanisms involved in the tolerant phenotype may not be conserved across all the models. STEPHEN R. KOCH's experiments show that in human endothelial cells, which contribute directly to innate immunity, priming by various TLR ligands can induce both tolerance and potentiation in the setting of secondary TLR challenges. With regards to endothelial cells, interferon-related chemokine production after LPS is weak compared to leukocytes. However, their data suggest that induction of IRFs, particularly IRF7, after Poly I:C priming allows for subsequent challenges via LPS to induce significant amounts of interferon-related chemokines. The most interesting result however occurred when examining TLR3 priming of TLR4-mediated IP-10 production, where Poly I:C priming greatly enhanced the IP-10/CXCL10 production in human umbilical vein endothelial cells by LPS. These studies provide new insight into the complexity of suggest that cross-modulation may lead to an impaired or enhanced inflammatory response depending on the stimuli.

In particular, in rapidly progressive or crescentic glomerulonephritis (RPGN), infiltrating CD4+ effector T cells of the Th1 and Th17 types release proinflammatory cytokines that directly promote tissue damage and stimulate chemokine production by renal resident cells, leading to the recruitment of additional leukocyte subsets and the subsequent loss of renal function. Among the mRNA analyzed, IP-10/CXCL10, which acts primarily via its receptor CXCR3 on activated T cells, was by far the most upregulated chemokine. Increased glomerular IP-10/CXCL10 expression was demonstrated by immunohistochemistry in human proliferative glomerulonephritis, IgA nephropathy, and crescentic glomerulonephritis. Our research also demonstrate that endothelial cell injury in acute and chronic glomerular lesions in patients with IgA nephropathy. But the relationship between them and the mechanism about the progression of IgA nephropathy is not release.

Our hypothesis is, glomerular endothelial cells with repeated LPS stimulated TLR3 priming highly express IP10,attractting inflammatory CCR3+ CD4T cell infiltration, causing tissue injury and progression of hematuria, albuminuria and renal insufficiency through cross-talk between endothelial, mesangial, podocyte, and parietal cells.

3)Materials and methods:

Animal model was set by HIGA mice injected LPS.LM/EM/SV-TEM/IHC/WB/q-PCR/FCM and co-immunoprecipitation was used for this experience.

4)Result:

After LPS interventioned for 9 weeks in HIGA mice, we successfully set a progressive IgA nephropathy model with significantly IgA deposit in messangial area and large albuminuria with/or without hematuria.

1．研究概要(2)

- 4.1 LM,EM,LV-SEM check histopathologic changes of glomeruli

Separation of CD34+ endothelial cells from the glomerular basement membrane and the loss of glomerular endothelial cells and diameter of the glomerular vascular loop, together with inflammatory cell infiltration, fibrin exudation, rupture of the glomerular basement　Separation of CD34+ endothelial cells from the glomerular basement membrane and the loss of glomerular endothelial cells and diameter of the glomerular vascular loop, together with inflammatory cell infiltration, fibrin exudation, rupture of the glomerular basement membrane, adhesion and/or crescent formation.

- 4.2) Correlations of acute glomerular lesions with proteinuria and hematuria

In the acute glomerular lesions, the presence of endocapillary hypercellularity, fibrinoid necrosis, and adhesion and/or cellular and fibrocellular crescents correlated with hematuria and huge proteinuria.

- 4.3 (future): Renal Chemokine and Chemokine Receptor mRNA and Protein Expression (including CXCL10/CCR2 in endothelial, mesangial cell and podocyte).

- 4.4 (future): The gap between endothelial cells and basement membrane was observed by electron microscopy. The inter-endothelial adhesion molecules and inflammatory pathways of endothelial cell injury include IL-6/JAK/STAT and NF/KB.

- 4.5 (future): Glomerular and blood lymphocyte types were analyzed by flow cytometry. Comparison of blood IgA -producing B cells and plasma pIgA/IgA and gdIgA1.

- 4.6 (future): Expression of podocytes specific markers such as mRNA expression of nephrin, podocin, and podoplanin and apoptotic proteins.

- 4.7 (future): Role of Endothelial-Mesangial-Podocytic Cross-Talk in IgA Nephropathy, the ultrastructural changes were carefully examined by electron microscopy. Special attention was paid to the expression of connexins between mesangial cells, podocytes and GBM, such as α3β1 ,αvβ3 integrin.

- 4.8 (future): Morphologic and Immunohistochemical Studies of IgA nephropathy with Acute Glomerular Lesions of Human.

- 4.9 (future): According to the understanding of the pathway mechanism, the intervention method was selected.

5) Discussion: IgA Nephropathy is usually a chronic and slowly progressive glomerulonephritis, but it can also manifest as a rapidly progressive GN. We successfully set the new model and hope to find out the mechanism and the way to deal with it.

6) 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							
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							
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論文名 3 Title							
掲載誌名 Published journal							
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その他著者名 Other authors							
論文名 4 Title							
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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				
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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	
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学会名 Conference				
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共同演者名 Co-presenter				
学会名 Conference				
演 題 Topic				
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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	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	
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） 清水 章

日中笹川医学奨学金制度(学位取得コース)中間評価書
論文博士：指導教官用

Supported by 日本財団 THE NIPPON FOUNDATION

第 43 期　研究者番号：G4306　作成日 ： 2023 年 3 月 10 日

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研究先（指導教官）	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)				
研究テーマ	食道癌に対する基礎的臨床的研究 Basic and clinical researches of esophageal cancer				
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	取得単位数 取得単位数／~~取得すべき~~単位総数
学生本人が行った研究の概要	1　病理学研究室にて臨床検体を用いた癌関連線維芽細胞からのオルガノイド作成 2　食道癌手術数と予後との関連を既報の論文を用いたメタアナリシスにて検討する 3　当科でのデータを用いて食道癌のリンパ節郭清個数と予後との関連を検討する。	
総合評価	【良かった点】 1　基礎的研究であり時間がかかっているが少しずつ進んでいる 2　Submit 中 3　論文作成中 以上のようにある程度研究は進んでいる	
	【改善すべき点】 大きな問題はないが、更に日本人スタッフらと密なコミュニケーションをとれるようにできると更によい。	
	【今後の展望】 1 については可能であれば論文作成までを目指す。 2，3　については accept を目指し、別のテーマについても解析する。	
学位取得見込	問題なく学位取得できるのではないかと考えている。	
評価者（指導教官名）	峯　王	

日中笹川医学奨学金制度(学位取得コース)中間報告書
研究者用

第43期　　研究者番号：G4306　　作成日：2023年3月10日

氏名	王 晴	WANG QING	性別	F	生年月日 1985/12/20
所属機関(役職)	中国医科大学附属第四医院胸部外科(主治医師)				
研究先(指導教官)	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)				
研究テーマ	食道癌に対する基礎的臨床的研究 Basic and clinical researches of esophageal cancer				
専攻種別	論文博士	☑	課程博士	☐	

1．研究概要(1)

1)目的(Goal)
Esophageal cancer is the eighth most commonly diagnosed cancer and the sixth most common cause of cancer death in the world[1]. In addition to esophagectomy, there are neoadjuvant chemotherapy, chemoradiotherapy molecular targeted therapy, immunotherapy, or a combination of modalities, but the prognosis for esophageal cancer is extremely poor, with a 5-year survival rate of less than 30%[2, 3]. Therefore, it is urgent to identify biomarkers that can predict treatment effects and to find new molecular targets.

Fibroblasts present in tumors are called cancer-associated fibroblasts (CAFs), which have been shown to promote cancer cell proliferation and malignant transformation[4]. Research on CAFs' function has been active in breast cancer, pancreatic cancer, and other areas, and some reports suggest that they contribute to tumor growth, metastasis, and treatment resistance in esophageal cancer[5].

In this study, we used immortalized fibroblasts to create CAFs using semi-artificial methods. CAFs produced by this method are called experimental CAFs, which have long-term stability and can be cultured on a large scale. They can also be used in in vitro co-culture experiments of cancer cells and CAFs, as well as in in vivo co-implantation experiments of cancer cells and CAFs. By creating these experimental CAFs and using them in various experiments, we will elucidate the characteristics and functions of CAFs in esophageal cancer. In addition to cancer cell lines, we also aim to create experimental CAFs that are more similar to in vivo CAFs by using cancer organoids in this study.

2)戦略(Approach)
To create experimental CAF and analyze it, two different types of cells (immortalized normal esophageal fibroblasts and patient-derived esophageal cancer organoids) were co-transplanted into immunodeficient mice.

3)材料と方法(Materials and methods)
①Immortalization of normal esophageal fibroblasts:
Immortalized fibroblasts are obtained by introducing the hTERT (telomerase) gene into fibroblasts derived from human esophagus. The fibroblasts are obtained from Cell Biologics and normal human esophagus specimens.

②Establishing patient-derived esophageal cancer organoids
Organoids derived from human esophageal cancer samples provide a novel and unique platform to model esophageal development, homeostatic regenerative differentiation, and benign and malignant esophageal diseases.

③Creation of experimental CAFs using esophageal cancer cells (cell lines/organoids):
CAF can be obtained by primary culture from surgical samples, but there are reports that their properties change after several passages and they become unstable, and it is difficult to use them as stable experimental materials due to cell aging. The method for creating experimental CAFs was developed in this course. This cell can be massively propagated and its properties are stable. Immortalized fibroblasts with antibiotic resistance are mixed with cancer cells and co-transplanted into immunodeficient mice to convert fibroblasts into CAFs within the tumor. Approximately 2 months later, the CAFs are isolated by removing the tumor and culturing it with antibiotics.

④Analysis using established experimental CAFs:
By comparing the gene expression of normal fibroblasts and established experimental CAFs, signal pathways that are upregulated in CAFs are identified, and the mechanism of CAF formation is predicted. In addition, functional evaluation is performed by mixing experimental CAFs with cancer cells and transplanting them into mice (to investigate tumor growth and cancer malignancy function).

1．研究概要(2)

4)実験結果(Results)fibroblasts.
①Immortalization of normal esophageal fibroblasts:
By detecting the population doubling level (PDL) of the cells, it was determined that the normal esophageal fibroblasts had become immortalized normal esophageal fibroblasts.

②Establishing patient-derived esophageal cancer organoids
Esophageal cancer organoids have been established using patient-derived esophageal cancer tissue. These are three-dimensional culture systems that can be grown in vitro to form miniaturized, self-organizing structures that mimic the architecture and function of the original tumor. These organoids are composed of different types of cells, including cancer cells, stromal cells, and immune cells, and can recapitulate the heterogeneity and complexity of the original tumor.

5)考察(Discussion)
①Why establish experimental cancer-associated fibroblasts (CAFs) instead of directly using CAFs from esophageal cancer patients?
Experimental cancer-associated fibroblasts (CAFs) are CAFs created using immortalized fibroblasts through semi-artificial methods. CAFs produced using this method have long-term stability and can be cultivated on a large scale. They can also be used in in vitro co-culture experiments of cancer cells and CAFs, as well as in in vivo co-implantation experiments of cancer cells and CAFs. By creating these experimental CAFs and using them in various experiments, we will elucidate the characteristics and functions of CAFs in esophageal cancer.
②Why establish patient-derived esophageal cancer organoids instead of using esophageal cancer cell lines?
Organoid technology can cultivate gastrointestinal tumors in a way that preserves their genetic, phenotypic, and behavioral characteristics, which is far superior to traditional tumor cell cultures[6].
③How to judge that normal esophageal fibroblasts have been immortalized?
By measuring population doubling (PD), it is more accurate to evaluate cell growth and determine if cells have undergone senescence[7].
6)参考文献(References)
1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-249.
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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	Association of Hospital Volume and Long-term Survival After Esophagectomy: A Systematic Review and Meta-Analysis					
掲載誌名 Published journal	The article is being submitted.					
	年 月	巻(号)	頁 ～ 頁	言語 Language		
第1著者名 First author	Qing Wang	第2著者名 Second author	Motomi Nasu	第3著者名 Third author	Tetsu Fukunaga	
その他著者名 Other authors	Shuko Nojiri, and Shinji Mine					
論文名 2 Title						
掲載誌名 Published journal						
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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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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 4 Title						
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その他著者名 Other authors						
論文名 5 Title						
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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さい

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

学会名 Conference	No									
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										
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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	No				
	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	
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） 峯　真司

日中笹川医学奨学金制度(学位取得コース)中間評価書
課程博士：指導教官用

第 43 期　　研究者番号：G4307　　作成日　：　2023 年 3 月 9 日

氏　名	張 瑛	ZHANG YING	性別	F	生年月日　1985/08/14
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研究先（指導教官）	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）				
研究テーマ	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 Ultrasound-based multi-modality imaging and therapy for hepatobiliary and pancreatic oncology and inflammatory bowel disease				
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 10／取得すべき単位総数 30
学生本人が行った研究の概要	初年度の必須講義は 10 単位分ですが、その分はすべて取得しました. 添付のように肝細胞癌診断における超音波と MRI 造影剤の意義の総説を記載し掲載された (Impact factor 5.738). (Ying Zhang, Kazushi Numata, Yuewu Du, Shin Maeda. Contrast agents for Hepatocellular carcinoma (HCC) imaging: value and progression. Frontiers in Oncology 2022 Jun 2;12:921667. 現在、肝細胞癌 BCLC stage B, C への局所治療と Lenvatinib での薬物治療の併用について propensity score matching を用いて解析し、局所治療を併用することの意義について評価中.	
総合評価	【良かった点】 総説が受理されたこと 肝細胞癌 BCLC stage B, C への局所治療と薬物治療の併用について propensity score matching を用いて解析中であるが有意義な結果がでており、患者さんの生命予後の延長に寄与できる. 【改善すべき点】 日本の若手医師とも交流をもってほしい. もっと指導医にざっくばらんに相談をしてほしい. もっと早く一緒に仕事をすすめていきましょう. 【今後の展望】 現在の研究をすすめて論文記載し投稿する 他の課題もすでにあるので、そちらも解析を実施し、論文作成までしていく予定	
学位取得見込	十分可能と考える	
評価者（指導教官名）　沼田和司　／ 前田慎		

日中笹川医学奨学金制度(学位取得コース)中間報告書
研究者用

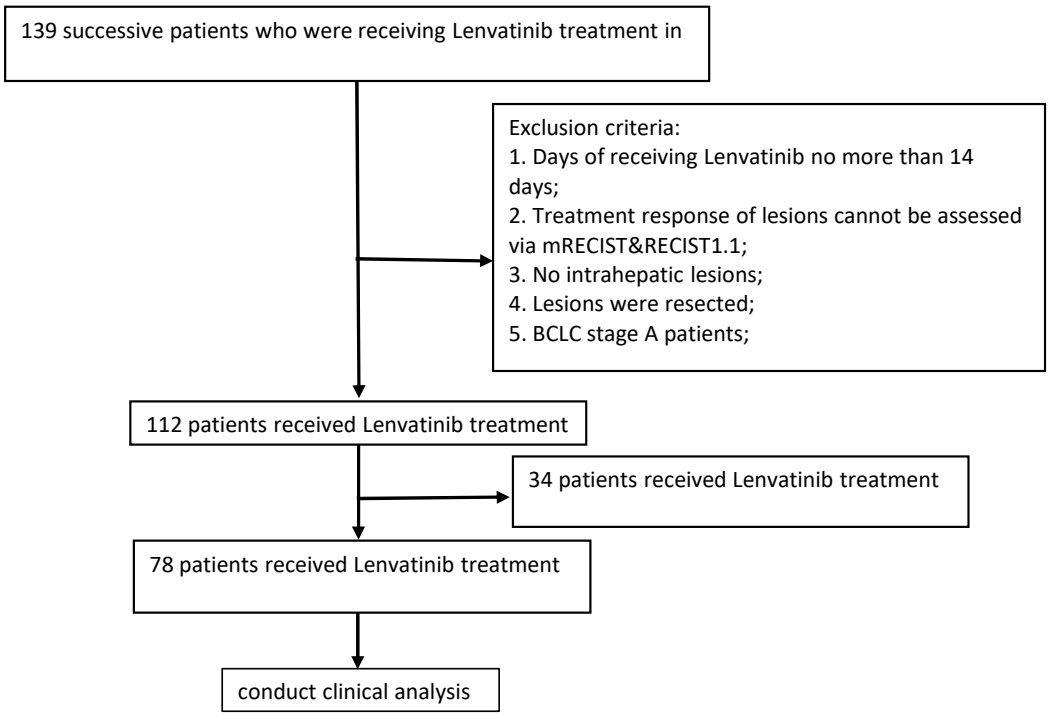
第43期　　研究者番号：G4307　　作成日：2023年3月9日

氏名	张 瑛	ZHANG YING	性別	F	生年月日 1985/08/14
所属機関(役職)	寧波市医療中心李恵利医院超音波科（主治医師）				
研究先（指導教官）	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）				
研究テーマ	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 Ultrasound-based multi-modality imaging and therapy for hepatobiliary and pancreatic oncology and inflammatory bowel disease				
専攻種別	論文博士	☐	課程博士	☑	

1．研究概要（1）

1）目的（Goal）
To explore the optimal use of lenvatinib in combination with locoregional therapies including RFA and to identify predictive biomarkers for patient selection.
2）戦略（Approach）
Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality worldwide. The BCLC staging system is widely used to guide treatment decisions for HCC patients and takes into account factors such as tumor size, number of nodules, presence of vascular invasion, liver function, and performance status. In patients with HCC, Barcelona Clinic Liver Cancer (BCLC) B and C stages are characterized by advanced tumor burden and limited treatment options. Lenvatinib is a potent inhibitor of multiple tyrosine kinase receptors, including vascular endothelial growth factor receptor (VEGFR) and fibroblast growth factor receptor (FGFR), which are critical in HCC tumor growth and angiogenesis. Several clinical trials have evaluated the efficacy and safety of lenvatinib in HCC patients with BCLC B or C stage disease. In a phase III trial, lenvatinib demonstrated a significant improvement in overall survival and progression-free survival compared to sorafenib, another tyrosine kinase inhibitor, in patients with unresectable HCC. lenvatinib has emerged as an important treatment option for HCC patients with BCLC B or C stage disease. Recently, it has been approved for the treatment of unresectable hepatocellular carcinoma (HCC) in patients with Barcelona Clinic Liver Cancer (BCLC) stage B or C. While lenvatinib has demonstrated efficacy in the treatment of advanced HCC, some patients may develop resistance to the drug over time. Like many other cancer treatments, lenvatinib can cause drug resistance, which means that the cancer cells may stop responding to the drug and continue to grow and spread. To overcome lenvatinib resistance, various strategies are being explored, including combination therapy with immune checkpoint inhibitors or other targeted therapies that target different signaling pathways, modification of the drug to improve its potency or reduce its side effects, and identification of biomarkers that can predict lenvatinib resistance and guide personalized treatment decisions. Meanwhile, recent studies have suggested that combination therapy with lenvatinib and other therapies may provide a promising treatment option for patients with HCC BCLC B and C stages. A phase II clinical trial conducted by Kudo, etal, showed the combination of lenvatinib and pembrolizumab with promising results, a higher objective response rate and longer progression-free survival compared to lenvatinib alone. Radiofrequency ablation (RFA) is a local ablative therapy that uses heat to destroy tumor cells, which has been well-tolerated with manageable adverse effects. Our previous research, a pilot study of combining Lenvatinib and RFA conducted by F Wang, eta al., indicated the inspiring results for intermediate-stage HCC patients. Given that, exploring the optimal use of lenvatinib in combination with locoregional therapies including RFA and identifying predictive biomarkers for patient selection are the indispensable needs, which are the main research theme of me.
4）実験結果（Results）
Fifty-four BCLC stage B and C HCC patients ranged from 53 – 90 years old were finally included in the present study, with 14 patients in the Lenvatinib-RFA sequential therapy group and 40 patients in the Lenvatinib monotherapy group. After starting Lenvatinib treatment, patients received RFA before progression is the Lenvatinib-RFA sequential therapy group. Otherwise, patients didn't receive any locoregional therapy after Lenvatinib starting is the Lenvatinib monotherapy group. After conducting propensity score matching to lower the confounding effects caused by covariates, the overall survival (OS, days) and progression free survival (PFS, days) were compared via Kaplan-Meier analysis with the p value of 0.037 (657.34+91.39 days vs. 918.16+89.23 days) and 0.006 (233.43+43.45 days vs. 591.11+81.54 days), respectively.
5）考察（Discussion）
The present retrospective cohort study was set to explore the promising solution for HCC patients with BCLC stage B and C when receiving Lenvatinib as the 1st line therapy. Based on the findings of the present study, it appears that the combination of Lenvatinib and locoregional therapy like RFA may provide a survival benefit for patients with HCC in BCLC B and C stages.(contiune on next page)

1．研究概要(2)
3)材料と方法(Materials and methods)



(contiune)

There have been several studies investigating the use of Lenvatinib for the treatment of HCC. A phase III trial published in the New England Journal of Medicine found that Lenvatinib provided a significant improvement in overall survival compared to sorafenib in patients with unresectable HCC. There have also been studies investigating the use of RFA for HCC treatment. A meta-analysis found that RFA provided a higher overall survival rate compared to other treatments for early-stage HCC. In terms of the combination of Lenvatinib and TACE, recent studies showed that the combination therapy provided a significant improvement in overall survival compared to Lenvatinib alone in patients with unresectable HCC. To sum up, findings of present study are consistent with prior research demonstrating the efficacy of Lenvatinib and RFA in the treatment of HCC. Further studies with extended sample size in multicenter will be needed to confirm the optimal treatment regimen for HCC patients in BCLC B and C stages.

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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	Contrast Agents for Hepatocellular Carcinoma Imaging: Value and Progression.					
掲載誌名 Published journal	Frontiers in Oncology					
	2022 年 6 月	12 巻(号)	NA 頁 ～ NA 頁	言語 Language	English	
第1著者名 First author	Zhang, Y.	第2著者名 Second author	Numata, K.	第3著者名 Third author	Du, Y.	
その他著者名 Other authors	Maeda, S					
論文名 2 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 3 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 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さい

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

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			
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	
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） 沼田和司 ・ 前田慎

frontiers | Frontiers in Oncology

REVIEW
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Contrast Agents for Hepatocellular Carcinoma Imaging: Value and Progression

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Hepatocellular carcinoma (HCC) has the third-highest incidence in cancers and has become one of the leading threats to cancer death. With the research on the etiological reasons for cirrhosis and HCC, early diagnosis has been placed great hope to form a favorable prognosis. Non-invasive medical imaging, including the associated contrast media (CM)-based enhancement scan, is taking charge of early diagnosis as mainstream. Meanwhile, it is notable that various CM with different advantages are playing an important role in the different imaging modalities, or even combined modalities. For both physicians and radiologists, it is necessary to know more about the proper imaging approach, along with the characteristic CM, for HCC diagnosis and treatment. Therefore, a summarized navigating map of CM commonly used in the clinic, along with ongoing work of agent research and potential seeded agents in the future, could be a needed practicable aid for HCC diagnosis and prognosis.

Keywords: ultrasound, MRI, CECT, hepatocellular carcinoma (HCC), contrast media (CM)

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INTRODUCTION

Hepatocellular carcinoma (HCC) has the third-highest incidence in cancers, along with the fourth leading cause of cancer death in 2020 globally. Moreover, cirrhosis, a major source of HCC, composed 2.4% of death with all causes in 2019 according to the WHO. Meanwhile, hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, alcohol abuse, and non-alcoholic steatohepatitis (NASH) are dominating etiological reasons for cirrhosis and HCC. Modern medicine believes the small HCC is preventable and curable through early diagnosis and timely etiological treatment if screening and surveillance could be well conducted for cirrhosis (1). Therefore, non-invasive medical imaging techniques, such as MRI, ultrasound (US), and CT, have contributed to HCC patients' management (2–6).

For early diagnosis, treatment assessment, and follow-up, multiple medical imaging modalities were improved and adapted in every corner of HCC prevention and supervision. In the past decades, the diagnostic efficacy of medical imaging has been elevated through the improvement of imaging resolution and associated intravenous contrast agents. US elastography and MR elastography are recommended to supervise and assess hepatic fibrosis, which may gradually progress to cirrhosis without medical intervention (7). On the other hand, taking characteristic advantage of the dual blood supply of the liver, transvenous contrast agents depict the liver lesion by illustrating the tumorous

blood supply with characteristics of arterial enhancement (wash-in) and portal hypodensity or hyposignal (wash-out). The classical imaging findings of wash-in and wash-out were believed to have a sensitivity of approximately 60% and a specificity of 96%–100% for small HCCs with a size of 10–20 mm. Still, a biopsy is needed in 40% of these lesions. Along with a deeper investigation of clinical research, an experienced radiologist can achieve a much more satisfying diagnostic efficacy through guidelines like the American College of Radiology Liver Imaging Reporting and Data System (ACR LIRADS) (8, 9). As a result, contrast enhancement imaging, like dynamic MRI and contrast-enhanced CT (CECT), is recommended in mainstream guidelines for preoperative HCC diagnosis with certainty. Screening using the non-enhanced US is also recommended for patients at a higher risk of HCC every 6 months. When it comes to contrast-enhanced US (CEUS), though it is not recommended by the World Federation for Ultrasound in Medicine and Biology (WFUMB) guidelines for liver lesion detection due to the narrow window for arterial phase observation (10), some meta-analyses indicated it to be a promising diagnostic approach for HCC with a sensitivity of 93% (95% CI: 91%–95%) and a specificity of 90% (95% CI: 88%–92%) (11), as well as the diagnostic efficacy of 93% in small HCCs (≦2 cm) (12).

Contrast-enhanced imaging for the tumor is a tracer technique of contrast media (CM) in essence. The distribution and dynamic phases of the agent are analyzed for lesion detection and characterization for early diagnosis and possible prognosis prediction. Therefore, a summarized navigating map of CM commonly used in the clinic, along with ongoing work of agent research and potential seeded agents in the future, could be a needed reference work for both physicians and radiologists.

BLOOD POOL CONTRAST AGENTS

Ultrasound Contrast Agents

As early as the late 1960s, people found that the microbubbles (MBs) that provide many reflecting interfaces for echo are a good intravascular flow tracer for US imaging (13), and the hydrogen peroxide solution was launched for echocardiography thereafter. According to the inner gas of the MB, US contrast agent (UCA) could be classified into two generations. Air core with the polymeric coat is the so-called first-generation UCA, such as Levovist (Schering, Berlin-Wedding, Germany). The first-generation UCA is a milestone in the history of medical US imaging development, though it comes with defects like unstableness and unsafety (13). Thereafter, inert gas that is enveloped with a lipid shell at a diameter of approximately several micrometers is developed as the second-generation UCA, which is slightly smaller than that of the red blood cell. Taking advantage of materials science and technology development, the second-generation UCA with greater stability and biosafety can achieve a promising diagnostic efficacy for HCC (11, 12), along with the negligible report of anaphylaxis compared with CT and MRI, which means that UCA can be employed for the patients having iodine allergy, chronic kidney disease, hepatic function failure, asthma, and so on. Moreover, the bedside operation with a portable US machine could be performed in the emergency department (ED) and intensive care unit (ICU) as needed. However, concerning clinical practice, CEUS is not good at imaging the hepatic lesion located near the lung and behind the costal bone, due to the so-called shadow zone caused by the costal bone and lung. The other weakness is US attenuation in far-field of a fatty liver can lead to the indefinable hepatic situation.

Currently, sulfur hexafluoride (i.e., SonoVue, Bracco Imaging, Milan, Italy) is the most consumed in the global UCA market, followed by perfluorinated butane (i.e., Sonazoid, GE Healthcare, Oslo, Norway). The former is a pure blood pool agent, while the latter behaves similarly at the beginning but permeates into extravascular space soon after administration, which will be discussed in Section 3.

Iodinated Agents for Contrast-Enhanced CT

Many iodinated agents are pure blood pool agents, which are the widest and longest used CM for X-ray-based enhancement scans (i.e., CECT) (**Figure 1**). To date, the effort of optimizing small-molecule iodinated agents for contrast enhancement could be mainly classified into three eras, including four categories of compounds, from ionic to non-ionic, from monomers to dimers, from high-osmolality to iso-/low-osmolality, associating with decreasing toxicity and increasing bio-tolerability. Commercially available agents are abundant in the clinic, such as iohexol (Omnipaque, GE Healthcare), iopromide (Ultravist, Bayer Healthcare, Leverkusen, Germany), iodixanol (Visipaque, GE Healthcare), iopamidol (Isovue, Bracco Imaging, Milan, Italy), and iothalamate (Cysto-Conray II, Mallinckrodt Imaging, St. Louis, MO, USA). Moreover, novel agents, like iosimenol and GE-145, are on the way to commercialization with the improvements made on an existing basis. The diagnostic efficacy of CECT for HCC in terms of area under the receiver operating characteristic (ROC) curve (AUC), sensitivity, and specificity were reported to be 0.93, 93%, and 82%, respectively (14). For HCC patients, the most distinctive role that CT perfusion imaging has played is the transarterial chemoembolization (TACE) assessment (15). However, despite great improvements that have been made in the bone and cartilage tissue, iodinated contrast agents employed in parenchymal organs, like the liver, have not yet been largely renovated (16, 17).

The blood pool agent applied to MRI is mainly established for MR angiography rather than the liver tumor, which is beyond the scope of the present review article and will not be discussed herein.

EXTRACELLULAR CONTRAST AGENTS

Non-Specific Agents

For MRI, gadolinium-based micromolecule agents that have five or seven unpaired electrons could be stimulated to be paramagnetic under an external magnetic field. Those so-called paramagnetic contrast agents for dynamic MRI are developed

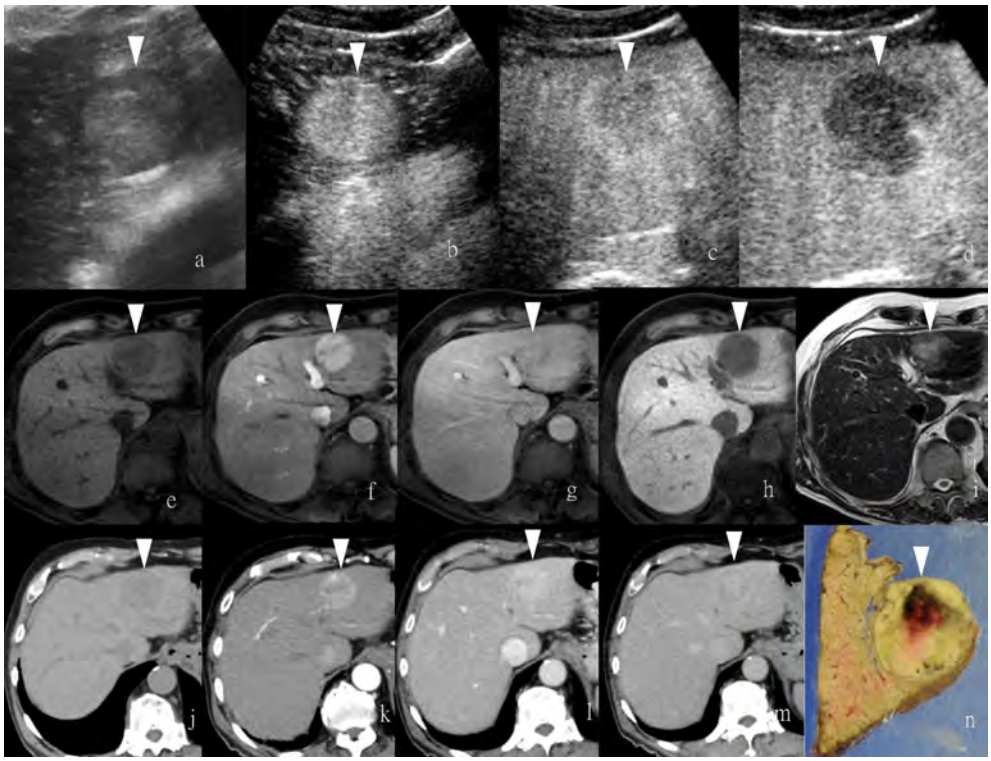

FIGURE 1 | Images of a man in his eighties with a pathological diagnosis of moderately differentiated hepatocellular carcinoma (HCC) and had a history of hepatitis **(C)** At the Sonazoid-enhanced ultrasound (US), the liver lesion at a size of 43 mm with a thin halo located at segment III was observed on B-mode US **(A)**. It was rapidly enhanced in the arterial phase (wash-in) **(B)**, started to fade (wash-out) in portal phase **(C)**, and was totally exhausted in the post-vascular phase **(D)**. At Gd-EOB-DTPA-enhanced MRI, the lesion was hypointense on T1-weighted image **(E)**, with the typical characteristics of wash-in and wash-out from arterial phase, portal phase, to delayed phase **(F–H)**. It showed hyperintensity on T2-weighted image **(I)**. At iodine agent-enhanced CT, it has low-density before enhancement **(J)**. It also showed wash-in and wash-out from arterial phase, portal phase, to delayed phase **(K–M)**. Finally, the gross specimen vividly reflected the morphological information of tumor **(N)**. Arrowheads indicate the margin of the HCC lesion.

and enriched (18). Gadolinium chelates (Gd-chelates) are clinically available mainstream for dynamic MRI on T1-weighted images, including Gd-DTPA (gadopentetic acid, Magnevist, Berlex, Berlin, Germany), Gd-DTPA-BMA (gadodiamide, Omniscan, Nycomed Amersham, Amersham, UK), Gd-HP-DO3A (gadoteridol, ProHance, Bracco Diagnostics, Milan, Italy), Gd-DTPA-BMEA (gadoversetamide, Optimark, Mallinckrodt, Staines-upon-Thames, UK), Gd-DOTA (gadoterate, meglumine, Dotarem Guerbet, Princeton, NJ, USA), and Gd-BT-DO3A (gadobutrol Gadovist, Schering Diagnostics, Berlin, Germany). These extracellular agents for non-specific liver MRI are commonly used worldwide because of the good patient tolerance and satisfying diagnostic efficacy (19). Thus, clinical recommendations from guidelines are almost based on the Gd-chelates (8, 9). Moreover, the informative images provided by contrast-enhanced MRI (CEMRI) also contribute to the therapy assessment (**Table 1**).

Reticuloendothelial System Endocytosis

Ferumoxytol, a kind of iron oxide nanoparticles (IONPs) approved by the Food and Drug Administration (FDA) as medicine for iron deficiency in adults, was recently reported to be feasible for MR angiography thanks to the characteristic of longer half-life in circulation and the advantage of superparamagnetism (20–23). The so-called negative contrast agents, containing iron oxide particles, darken the normal liver background on T2-weighted images to negatively enhance the target issue, in contrast with the so-called positive agents that brighten the target tissue on T1-weighted images, like Gd-chelates. The first commercially available reticuloendothelial system (RES)-specific contrast agent is ferumoxides (Feridex) (24), which makes lesions that contain negligible RES cells conspicuous on T2-weighted images since the normal liver background containing many RES cells can selectively take up iron oxide particulates to lower the T2 signal intensity (25). Iron oxide crystals coated with dextran or carboxydextran are named superparamagnetic iron oxide (SPIO), which is normally employed as T2 MR CM. With a sufficient infusion of SPIO, normal hepatocytes containing many Kupffer cells are supposed to catch most SPIO particles, leading to a dark area on T2-weighted images. By contrast, tumors, whether benign or malignant, primary or metastatic, that are deficient in Kupffer cells cannot exhibit SPIO uptake, shaping a relatively hyperintense area. However, focal nodular hyperplasia (FNH)

TABLE 1 | The categories of extracellular contrast agents in clinical practice.

Category	Specificity	Class	Classical agents	Featured purposes	Modality
Extracellular agent	Non-specific	Gadolinium chelates	Gadopentetic acid (Gd-DTPA)	Tumor imaging; blood pool imaging	T1 agent for MRI
Reticuloendothelial system (RES) agent (Kupffer cells included)	RES specific	Iron oxide	Ferucarbotran (Feridex)	Liver tumor imaging	T2 agent for MRI
		Microbubbles	Perfluorinated butane (Sonazoid)	Liver tumor imaging; blood pool imaging	Ultrasound contrast agent
Hepatobiliary agent	Hepatobiliary specific	Manganese-based compound	Mangafodipir (Mn-DPDP)	MR cholangiography; liver function indicator	T1 agent for MRI
			Gadobenate dimeglumine (Gd-BOPTA); gadoxetic acid (Gd-EOB-DTPA)	Liver tumor imaging	T1 agent for MRI

seems to be an exception, since SPIO particles may accumulate there and lead to a resultant isointense or even hypointense appearance (26, 27). Following SPIO, the derivative in terms of ultrasmall particulate iron oxides (USPIO) with advantages of convenient administration and striking prolonged plasma half-life that enables it also as a blood pool agent was developed thereafter (28, 29) (**Table 1**).

Regarding UCA, Sonazoid is an MB of perfluorobutane core wrapped by the shell of hydrogenated egg phosphatidylserine. At first, Sonazoid MBs were used as the blood pool contrast agent. As early as 1 min after the intravenous administration, the MBs start to diffuse into extravascular and intercellular space where they will be phagocytosed by the Kupffer cells in the normal liver sinusoids. Approximately 10 min later, once intravascular MBs are mostly eliminated, the remaining stable MBs endocytosed by resident macrophages in liver parenchyma will shape the so-called additional Kupffer phase or post-vascular phase, which can last to 2 h after injection (30–32) (**Table 1**). Moreover, in the classical enhancement features of wash-in and wash-out, HCC theoretically appears to be perfusion defects in the Kupffer phase or post-vascular phase because of Kupffer cell shortage (**Figures 1**, **2**). The characteristics of the additional post-vascular phase aid much in HCC detection and diagnosis. Recently, Sonazoid has been proven to be non-inferior to SonoVue in a retrospective clinical study for focal liver lesion (FLL) (33). However, if the lesion is isoechoic in the post-vascular phase, misdiagnosis can happen at a rate of approximately 17% (34). Worse still, owing to histological reasons of some well-differentiated HCC, the sign of perfusion defect in the Kupffer phase could be observed at a rate of only 69% among HCC patients (35). Also, some benign lesions that lack Kupffer cells have a chance to be misdiagnosed as a false-positive sign in the Kupffer phase (36). Therefore, the expected additional clinical benefit on diagnosis gained from the Kupffer phase has not yet been confirmed (37). As for HCC intervention, after US brings real-time monitoring for minimally invasive operations like lesion biopsy and regional ablation, CEUS is employed for more accurate guidance and unique immediate evaluation during therapy (38–43). Vascular-sensitive assessment makes CEUS an indispensable aid for effective

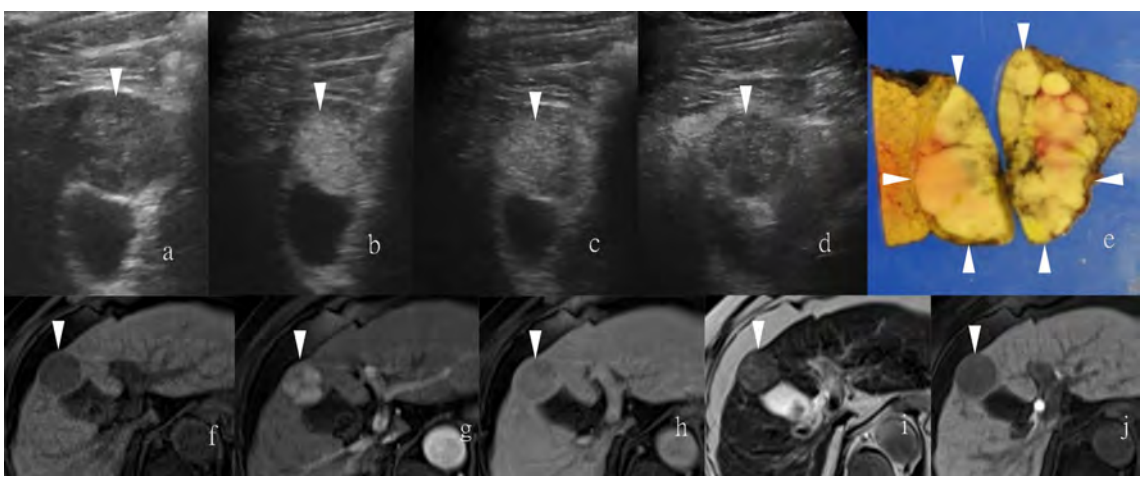

FIGURE 2 | Images of a man in his sixties with a pathological diagnosis of poorly to moderately differentiated hepatocellular carcinoma (HCC) and had a history of cirrhosis. At the Sonazoid-enhanced ultrasound (US), the liver lesion was heterogeneous hyperechoic with the indistinct margin on B-mode US **(A)**. It was rapidly enhanced in the arterial phase (wash-in) **(B)**, still iso-echoic in portal phase **(C)**, and was totally exhausted in the post-vascular phase **(D)**. At Gd-EOB-DTPA-enhanced MRI, the lesion was hypointense on T1-weighted image **(F)**, with uncharacteristic wash-in and delayed wash-out from arterial phase to delayed phase **(G, H)**. It showed hyperintensity on T2-weighted image **(I)**. The contrast media (CM) were totally exhausted till the hepatobiliary phase **(J)**. The gross specimen indicated the heterogeneous pathological differentiation of HCC **(E)**. Arrowheads indicate the margin of the HCC lesion.

radiofrequency (RF)/microwave (MV) ablation (44, 45). On the other hand, three-dimensional (3D) US can provide additional lateral and other viewing angles, and morphological information offers UCA another usable imaging modality (i.e., contrast-enhanced 3D US, CE 3D US) (46, 47) (**Figure 3**). Moreover, contrast enhancement is also employed in fusion imaging to reveal extra small liver lesions and biopsy navigation (48) (**Figure 4**).

Hepatocyte-Specific Uptake

Mangafodipir trisodium (Mn-DPDP) used to be a classical hepatocyte-selective contrast agent that was developed in the last century and has favorable contrast-to-noise measurements and lesion detection rate as compared to non-enhanced MRI (49, 50). It was high-profile at the beginning for the prolonged enhancement relative to the traditional T1 contrast agents (51). The uptake of Mn-DPDP occurs in hepatocytes, and its elimination is in the biliary tree. Thus, the metabolism process of Mn-DPDP can indicate hepatobiliary function (52, 53). Moreover, it is reported that the hepatocyte-selective contrast agent is correlative with the pathological differentiation degree of HCC (54). Since the uptake of Mn-DPDP strictly occurs in hepatocytes, the extrahepatic originated metastases can be negatively illustrated (55). However, in contrast to the question of how many normal hepatocytes are contained in a lesion, the question of whether a liver lesion is malignant or not will be the highest concern for patients.

By integrating the mechanisms of both hepatocyte-selective contrast agents and non-specific extracellular Gd-chelates, gadolinium-based hepatobiliary-specific agents were thereby developed, such as gadobenate dimeglumine (Gd-BOPTA) and gadoxetic acid (Gd-EOB-DTPA), which are worldwide commercially available and have become a promising MRI contrast agent for FLL (56–58). For HCC diagnostic imaging, the so-called hepatobiliary contrast agents achieve further detection in the early stage for primary, recurrent, and metastatic HCCs through usual dynamic imaging and additional hepatobiliary delayed phase (59–62) (**Figures 1**, **2**). Beyond diagnosis, uptake of Gd-EOB-DTPA of HCC lesions is reported to be a biomarker for prognosis (63), as well as the estimation of liver function (64). Concerning patients' tolerance, Gd-EOB-DTPA only requires a minimum injection dose to present a satisfying enhancement in the liver and smaller branch of the biliary tree relative to Gd-BOPTA (55) (**Table 1**).

MOLECULAR IMAGING AGENTS

For the diagnostic and therapeutic purpose of molecular imaging, by means of conjugating some antibody, peptide, or ligand, molecular imaging agents are artificially designed to anchor the targeted cellular and molecular hallmarks pathologically (65).

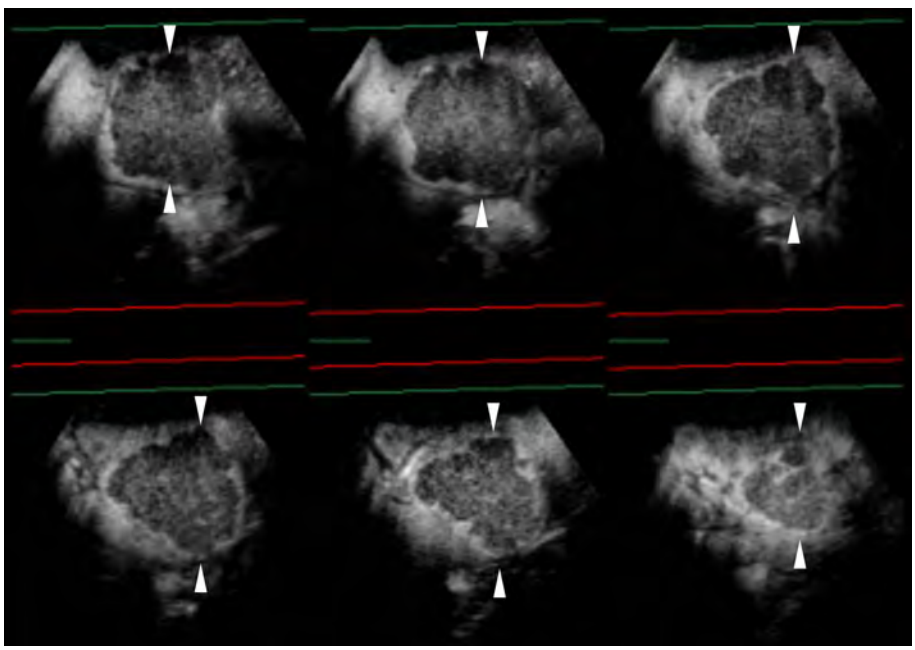

FIGURE 3 | Sonazoid-enhanced ultrasound (US) images of a man in his seventies with a pathological diagnosis of moderately differentiated hepatocellular carcinoma (HCC), who had a history of hepatitis C. The tumor was 70mm. Consecutive lateral images of the tumor remarkably illumed the irregular margin on the three-dimensional (3D) US, which was obtained by auto-sweep 3D scanning in the post-vascular phase. Tomographic ultrasound images in plane A, which can be translated from front to rear, with a slice distance of 4.8 mm. Arrowheads indicate the margin of the HCC lesion.

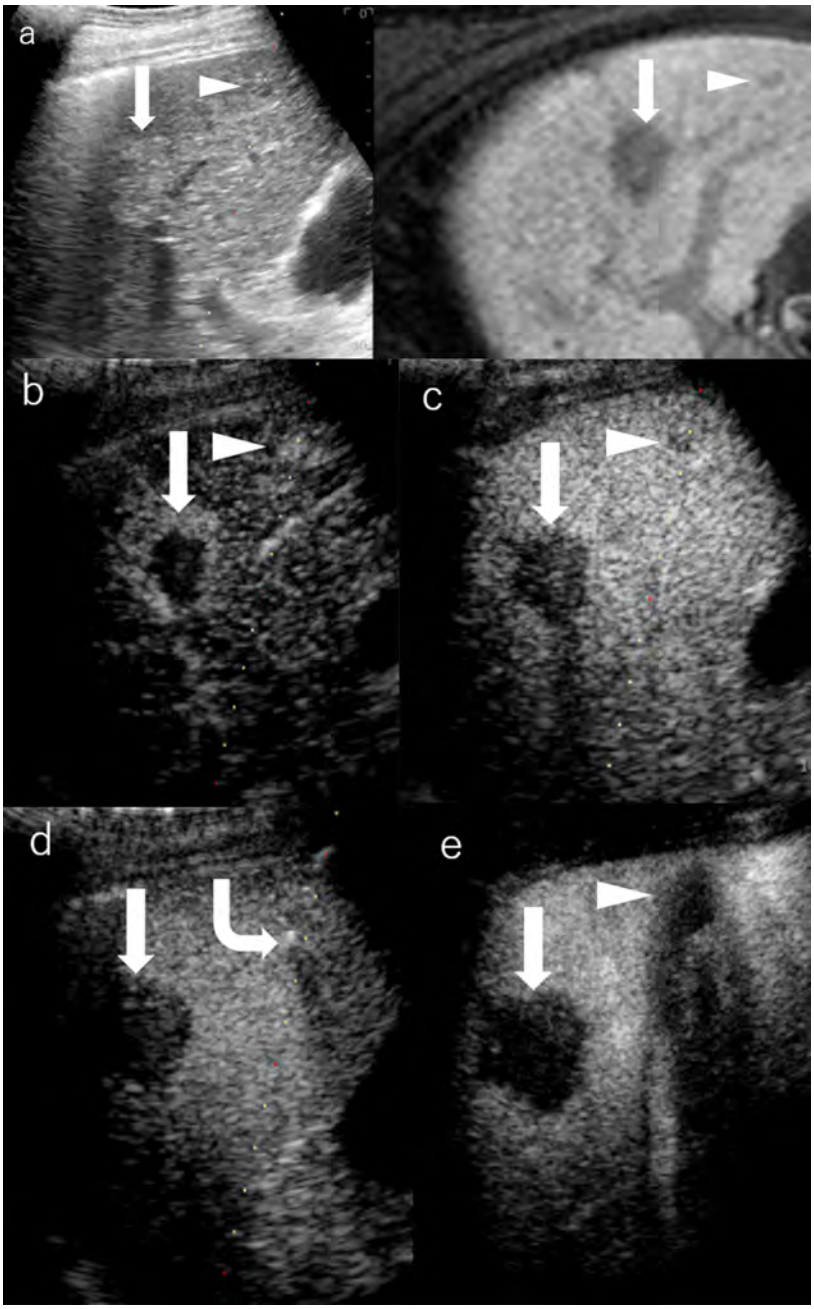

FIGURE 4 | Images of a man in his seventies with a pathological diagnosis of moderately differentiated hepatocellular carcinoma (HCC) and had a history of cirrhosis and HCC. The hepatobiliary phase of EOBMRI (right side), as the reference, was combined with conventional grayscale US (left side), displayed an 8-mm indistinctive hypointense area (the triangular arrow) in segment V on the same screen for the fusion imaging **(A)**. The extrasmall lesion was hypervascular in the arterial phase of Sonazoid-enhanced ultrasound (US) **(B)**, while the post-vascular phase indicated it to be a slightly hypoechoic area **(C)**. Pathway guidance was ready for radiofrequency ablation (RFA) needle manipulation on real-time US **(B–D)**, along with tracking for the metallic needle tip (the curved arrow) **(D)**. The contrast-enhanced US (CEUS) evaluated the target ablation area to be non-enhanced after RFA **(E)**. Arrows indicate the margin of the bigger HCC lesion, which was previously treated by RFA. And Arrowheads indicate the margin of the extrasmall HCC lesion.

Immune Molecular Anchoring

By means of immunoreaction, gadolinium-labeled reagents for liver tumor marking and monitoring of the MR modality are commonly employed in a tumor-bearing animal model for cancer research (66, 67). The molecular weight of reagents mainly ranges from dozens to hundreds of kDa. Likewise, the MBs or nanobubbles binding compounds marked with the tumor-specific immune molecule are also available for cancer research in the CEUS modality (66).

Stimulus-Responsive/Microenvironment-Dependent Contrast Agents

A T1/T2 switchable MR contrast agent was recently validated on a mouse model for HCC early diagnosis (68, 69). Previously, the diagnostic efficacy of IONP-based MRI was not as high as expected when it was simply employed as a liver-specific T2 agent (70). However, researchers recently found that IONP clusters could be accordingly disaggregated thanks to the acidic tumor microenvironment, which can generate a downstream tumor-specific T1 contrast agent. As a result, the IONP agents can additionally be employed to delineate HCC on T1-weighted images after switching to a downstream tumor-specific contrast agent. Based on IONP, agents decorated with functional small-molecular ligands through surface engineering are thereafter designed to be stimulus-responsive agents, pH-sensitive, and nanoscale distance-dependent (68, 71–75). Furthermore, concerning the aggregation phenomenon that commonly happened in nanoparticles with a large surface area/volume ratio, ultrafine nanoparticles could facilitate intratumoral homogeneous distribution of contrast agents (76). IONP at a diameter of 3.6 nm is supposed to be an optimal T1 agent *in vivo* (77). Moreover, core engineering of various designs of size, shape, composition, surface coating, molecular weight, and drug delivery has indicated IONP to be a hopeful T1 contrast agent (78–85). Beyond imaging, Yang et al. developed a novel nanoparticle that releases Fe^{2+} for the treatment of folic acid (FA) receptor-positive solid tumors through the ferroptosis pathway while being supervised through the Mn agent-enhanced imaging (86, 87). Also, Song et al. developed an assay of therapeutic natural killer cells (NK cells) conjugated with Sonazoid MB to make the antitumor process visible in real-time CEUS (88).

Scale-Dependent Particles

As nanomedicine was developed recently, emerging nanomaterials have been studied for contrast enhancement imaging. Some nanoscaled CM can permeate into tumor stroma through weak tumor vessels to depict the tumor with or without the assistance from functional parts equipped in advance (89). Moreover, sonoporation induced by external stimulation of focused US can reversibly increase the permeabilization of the cell membrane, leading to the potential visualization of HCC intracellular therapy in the future (90).

CLINICAL CHALLENGES AND PROSPECTS

As for the clinically commonly used contrast agents, Guang et al. performed a meta-analysis to compare the diagnostic value of CEUS, CT, and MRI in FLL. To rule out HCC from FLL, CECT has the highest sensitivity of 90% (95% CI: 88%–92%), followed by CEUS (88%) and CEMRI (86%). Both CEUS and CEMRI have a higher sensitivity of 81% than CECT (77%). However, all results have no statistical significance (16, 91). Moreover, Westwood et al. found that CEUS could be a cost-effective alternative for HCC diagnosis relative to CECT or CEMRI with similar diagnostic performance (92). Research about combined multimodal medical imaging (including Sonazoid-enhanced US, Gd-EOB-DTPA-enhanced MRI, and CECT) conducted by Masatoshi Kudo figured out that the sensitivity for HCC diagnosis is 72%, 74%, and 86% for CEUS, CECT, and Gd-EOB-DTPA-enhanced MRI, respectively, with no significance among the three imaging modalities. When combining US with MRI, the sensitivity soared as high as 90% (93).

Meanwhile, controversies still remain regarding the diagnostic efficacy of HCC. Despite that the hepatobiliary agent-enhanced MRI is believed to reach an early diagnosis for HCC that is still in the hypovascular stage (94), researchers analyzed the clinical trials that use different contrast agents for HCC diagnosis and found no significant difference in the diagnostic efficacy in terms of sensitivity and specificity between the MRI using extracellular agents and hepatobiliary agents (95, 96). Imbriaco et al. claimed that Gd-EOB-DTPA-enhanced MRI has a better diagnostic performance than CECT only for lesions that are smaller than 20 mm and patients with Child-Pugh class A (97). Moreover, for patients with cirrhosis, Kim et al. demonstrated better performance of hepatobiliary agent-enhanced MRI relative to routine US screening for surveillance of people at a higher risk of HCC (2). In addition, molecular imaging agents, like IONP-based MR agents, are still on the way to fulfilling the various clinical needs (98). On the other hand, although current CM has been deeply improved through materials science, biosafety is still the most crucial factor for patients having various allergies and metabolism troubles. Necessary reinjection of contrast agents for CT and MRI may come with a potential risk of side effects. Minimized dose of contrast agent that meets all clinical needs will be a future trend for CM research.

To sum up, the CM brings out the best diagnostic performance for suitable patients under appropriate conditions. Although Gd-DTPA-enhanced MRI and non-ionic iodinated agents-enhanced CT are usually recommended for HCC diagnosis by mainstream guidelines, liver-specific CM, like Gd-EOB-DTPA and Sonazoid, have already played an anticipated role in HCC diagnosis and prognosis prediction. Furthermore, the amelioration of molecular imaging agents has drawn a blueprint for future medical imaging.

AUTHOR CONTRIBUTIONS

Concept and design: KN and YZ. Manuscript writing: YZ. Figure presentation: KN. Reviewed the manuscript: all authors. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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課程博士：指導教官用

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成績状況	㊧　良　可　不可 学業成績係数=	取得単位数 ４／４
学生本人が行った研究の概要	von Willebrand 因子（VWF）は止血を担う血漿タンパク質であり、ADAMTS13 は VWF を切断して止血機能を抑制するプロテアーゼである。VWF と ADAMTS13 の機能的バランスが崩れると出血性あるいは血栓性の疾患につながる。von Willebrand 病は VWF の遺伝子異常に伴う出血性疾患である。その診断や病型分類のためには遺伝子解析が重要であるが、VWF 遺伝子の特徴から、従来の解析方法では実施のハードルが高い。そこで、新しいロングリードシーケンシング法を工夫して VWF 遺伝子を効率よくかつ正確に解析することを目標とした。今年度は、VWF 遺伝子全体の配列データ取得と偽遺伝子除外により遺伝子異常を正確に同定するためのワークフローを構築した。	
総合評価	【良かった点】 研究に対する熱意はきわめて高く、常に向上心を持って取り組む姿勢が見える。VWF 遺伝子全長の PCR とロングリードシーケンシングの実験条件検討にはかなりの労力を要することとなったが、目標達成のために日々の実験計画をしっかり立て、アドバイスを受け入れながら実験を行い、着々と研究を進めた。 【改善すべき点】 新しい技術を習得する際、ミスを避けたいという気持ちが強いため、過度に慎重になることがある。慎重さが重要であることは言うまでもないが、キーポイントを正確に把握することで、もう少し大胆に、気持ちに余裕を持って実験を行うことができると、研究能力がより伸びると思われる。 【今後の展望】 次年度は、今年度に確立したロングリードシーケンシングによる解析結果をサンガー法で検証する。さらに、同定した遺伝子バリアントがもたらす影響を、患者血漿の解析と培養細胞による発現実験解析で調べ、病態との関連を明らかにする。	
学位取得見込	今年度の計画は順調に進んだ。来年度の実験のための準備も進んでいるため、目標期間内に学位を取得できる見込みは大きい。	
評価者（指導教官名）　中川 修		

日中笹川医学奨学金制度(学位取得コース)中間報告書
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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 a genomic analysis approach using long-read sequencing and validate the effectiveness on identification of causative variants in von Willebrand factor (VWF) gene responsible for von Willebrand disease (VWD).

2）戦略（Approach）

In this study, we established a novel genetic analysis workflow in VWD or Acquired von Willebrand syndrome (AVWS) by Oxford Nanopore sequencing technology (ONT) which can discover not only the long structural genetic defects, but also the single nucleotide variants with high accuracy and fast speed [1-3].

Firstly, using genomic DNA samples from healthy donors, primer pairs used to amplify PCR amplicons covering entire VWF were designed and optimized according to the results of long-range PCR and following agarose gel electrophoresis to avoid nonspecific amplification due to repetitive sequences or pseudogene VWFP1 [4]. Then, using the most optimal PCR protocols, DNA samples from VWD or AVWS patients were amplified, all PCR amplicons were purified and prepared for ONT sequencing. ONT data was then analyzed using the corresponding software and variants on both exons and introns were called and investigated. All identified causative mutations were verified by Sanger sequencing.

3）材料と方法（Materials and methods）

Patients

Two patients from the National Cardiovascular Center (NCVC) Biobank with VWD or AVWS were enrolled in our study and informed consents were obtained. The patients were classified by laboratory and clinical investigation results based on JSTH VWD guideline [5].

DNA samples

Genomic DNA samples provided by NCVC biobank were isolated from peripheral whole blood. DNA purity and concentration was determined and standardized to 50ng/μL.

Long-range PCR and ONT sequencing

PCR primer pairs used to amplify 185kb sequence from 5kb upstream to 5kb downstream of VWF gene were designed by Primer-Blast on NIH. Each PCR reaction mix consisted of 0.2 μM each of the forward and reverse primer, 1× PrimeSTAR GXL Buffer, 0.2 mM dNTP mix, 100ng-200ng genomic DNA, and 1.25 units PrimeSTAR GXL DNA Polymerase, combined in a 50μl reaction (Takara Bio, Shiga, Japan). The PCR cycling conditions were as follows: initial denaturation of 94ºC for 2min, followed by 30 cycles of 98ºC for 10s, 68ºC for 10min, followed by a final 68ºC extension for 5min. All PCR amplicons were verified by 1.2% agarose gel electrophoresis then purified using Ampure XP magnetic beads (Beckman Coulter, California, USA) and quantified by Qubit Fluorometric Quantification Broad Range Assay (Invitrogen, California, USA).

Then, the final 20 fmol DNA library was prepared for GridION sequencing using the ligation kit (SQK-LSK114) in accordance with the manufacturer's instructions (Oxford Nanopore technology, Oxford, UK).

ONT data analysis

Library samples were sequenced on a single R10.4 flow-cell in 6-12h run using the FLO_MIN114_SQK-LSK114 protocol and MinKNOW v22.12.5. Base-calling was carried out using Guppy v6.4.6 with high-accuracy model.

Reads with quality <9 and length <8000 nt or >16000 nt were discarded using filterlong v0.2.1. After mapping reads with minimap2 v2.24 to hg38 region chr12:5,944,046-chr12:6,128,632 for alignment to produce BAM files, variants were called using the Clair3 v0.1-r12 and Longshot v0.4.5. WhatsHap v1.6 was used to phase the heterozygous variants stored in the ONT VCF files, relying on the information included in the BAM file.

All SNV/INDEL allele genotype frequency and other details such as count of homozygous individuals or SNP number were investigated by 38KJPN database on jMorp (https://jmorp.megabank.tohoku.ac.jp).

For candidate SNV mutations, dbSNP (http://www.ncbi.nlm.nih.gov/SNP) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) through NCBI was used to check the variant details and clinical significance. ISTH VWF Database(http://vwf.hemobase.com) were also checked to see if the variant had been previously recorded or reported.

1．研究概要(2)

4)実験結果(Results)

Optimization of VWF amplification using long-range PCR

To amplify whole VWF gene without undesirable non-specific amplicons from repetitive sequence or pseudogene VWFP1, long-range PCR was performed using different custom-designed primer pairs [6]. According to the agarose check followed, some primer pairs with low efficiency were redesigned, and after several optimizations, forty-two primers were determined, and twenty-one 12kb-15kb amplicons covering VWF gene were produced despite yielding some minor nonspecific bands under 10kb.

Variants calling using Clair3 and Longshot

Two samples of patients were amplified using the optimal PCR protocol and sequenced on R10.4 flow-cell, generating 95.38k reads for 6 hours and 128.13k reads for 12 hours, respectively. Variants calling was performed by Clair3 and Longshot using ONT data, and all candidates were output into two lists with 325 and 305 called variants, respectively. One missense SNV variant in each sample was found having extreme low allele frequency under 1.80% in Japanese population and not been recorded or reported yet. For the functional validation, we are planning to perform VWF multimer analysis using patient's plasma and the expression experiment in cultured cells using VWF gene with these two variants idengtified before.

5)考察(Discussion)

Considering the complexity of 175kb VWF gene contains 52 exons with multiple repetitive sequences, and the presence of a 21-29kb pseudogene VWFP1, Sanger sequencing or short-read next-generation sequencing (NGS) commonly used are not available in VWF on most occasions [4]. Hence, ONT with long-read sequencing ability that can overcome these difficulties was utilized in our study. Since the availability of it have been proven [1-3], to establish a practical and feasible methodology combining it and long-range PCR in VWD investigation, we paid more attention on developing a PCR approach that enable to (1) amplify 10-15kb PCR products reliably with high fidelity and specificity, and (2) amplify regions that is currently difficult for Sanger and NGS, and (3) provide favorable PCR products for following ONT sequencing as templates.

For long-range PCR, Invitrogen Platinum SuperFi II DNA Polymerase which featuring exceptional >300X Taq fidelity, high sensitivity, and specificity as described in manufacturer's instruction was firstly used. Unfortunately, although the application was successful in other study [7], it only produced smeared and weaker 15kb products with some nonspecific bands <10kb. After several failed attempts, we tried another polymerase also developed for long-range PCR, TaKaRa PrimeSTAR GXL DNA Polymerase, and it was found can amplify clear and strong bands from VWF gene in our study.

To overcome the obstruction to PCR brought by multiple repetitive sequences on VWF, we designed the primers with repeat filter on and exclude all primers may contain known SNP on binding site, because once we cancel the filter or accept those contain SNP, specific DNA products were not obtained in most cases. For regions could not be generated by primer pairs meet the requirements mentioned above, target sequence would be replaced by two or three 15k fragments which can be produced each by primer pairs with low repeat and non-SNP to cover the original region. The pseudogene VWFP1 located on chromosome 22 corresponds to 12 exons (exon 23-34) of VWF gene, shows approximately 97% homology. Basing on four VWF-selective primers verified by Mancuso et al. [6], other four primers were designed and the specificity of those 15kb products was also confirmed by the agarose gel check and the following ONT sequencing.

Even using workable polymerase and high-specific primer pairs, it still took a long time to determine the suitable template amount. Some study used genomic DNA of 35ng in 20μl reaction or 0.25ng-6ng in 12.5μl reaction to evaluate some enzymes for long-range PCR, and PrimeSTAR GXL produced amplicons ranged from 5.7kb to 13.7kb successfully [8-9]. However, similar results were not repeated in our study neither in 20μl nor 50μl reaction. Therefore, PCR with different amounts of template were performed, and 100ng-200ng was considered be the optimal genomic DNA input in 50μl reaction and used afterwards.

Given most VWD is autosomal dominantly inherited, one rare mutation identified by ONT in our study was sufficient to explain the symptom and diagnosis of the patient. For AVWS, although it is not an inherited disorder pathogenetically, the missense variant discovered may represent a potential genetic risk factor masked by the primary underlying diseases.

In general, we reported a novel genetic analysis method in VWF-related hemorrhagic disease by long PCR and Nanopore sequencing which could be a powerful tool in investigating the pathogenetic mechanisms of VWD or AVWS on a molecular level in near future.

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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						
掲載誌名 Published journal						
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学会名 Conference				
演　題 Topic				
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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	
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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	
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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)

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	国名 Country		受賞年 Year of	年　　月
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	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
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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
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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			

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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） 中川　修

日中笹川医学奨学金制度(学位取得コース)中間評価書
課程博士：指導教官用

第 43 期　　研究者番号：G4310　　作成日　：2023 年 3 月 1 日

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研究テーマ	**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** Identification and functional analysis of host factors that regulate SARS-CoV-2 replication				
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=	取得単位数 取得単位数／取得すべき単位総数
学生本人が行った研究の概要	ウイルスの複製に関わる宿主因子の研究は、特にレトロウイルス学において最先端で進んでいることから、まず HIV の複製を阻害する抗ウイルス宿主因子の分子ウイルス学的研究に着手した。HIV-1 エンベロープ糖タンパク質（HIV-1 Env）および水胞性口炎ウイルス G タンパク質（VSV-G）に対する動物種別 MARCH8 の抑制効果について検討した。サル（Rhesus macaque）、マウス、ウシの MARCH8 野生型および 2 種類の変異体（RING-CH 変異体とチロシンモチーフ変異体）を作製した。これらのウイルスエンベロープ抑制活性を検討するため、各 MARCH8 を HIV-1 Env 欠損型ルシフェラーゼレポーターウイルス DNA、そして HIV-1 Env 発現プラスミドまたは VSV-G 発現プラスミドと共にヒト胎児腎細胞株 293T にコトランスフェクションして、産生されたウイルスの感染性を検討した。その結果、ヒト MARCH8 と同様、サル、マウス、およびウシ MARCH8 の野生型は HIV-1 Env と VSV-G に対する抑制能を保持しており、一方で 2 種類の変異体はどちらも抑制活性を失っていた。このことから MARCH8 の抗ウイルスエンベロープ活性およびその機能領域は動物種間で高度に保存されていることが明らかになった。	
総合評価	【良かった点】 ・ウイルス学研究に対するモチベーションが高い。 ・真摯に実験に取り組み、失敗しても諦めずに何度もやり直す姿勢を持っている。 ・関連研究に関する情報を収集するべく、最新論文のチェックを細目に行っている。 【改善すべき点】 ・最初に研究指導を受ける際にメモを取りノートにまとめる習慣をつける。 ・試薬/消耗品を使い切って初めて発注要求したりせず、事前に誰かに伝える。 ・初心者のうちは、トラブルシューティングを全て自分で行おうとせず、悩む前に指導教官に相談することを心掛ける。 【今後の展望】 前述した【改善すべき点】に十分留意して日々の実験を行っていけば、徐々に良いデータを蓄積して、学位論文を書き始めることが出来るのではないかと期待している。	
学位取得見込	医学博士課程の 4 年間のうち、コロナ禍による入国制限のために最初の 13 カ月半をすでにロスしており、残りの年月で実験の修得と実施、学位論文の作成・投稿・採択まで全て終えることはチャレンジングではあるが、指導教官として最善を尽くし期限内に学位を取得させたい。	
評価者（指導教官名）　徳永　研三		

日中笹川医学奨学金制度(学位取得コース)中間報告書
研究者用

Supported by 日本財団 THE NIPPON FOUNDATION

第43期　　研究者番号：G4310　　作成日：2023年3月　1日

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研究テーマ	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 Identification and functional analysis of host factors that regulate SARS-CoV-2 replication				
専攻種別	論文博士	□	課程博士	☑	

1．研究概要（1）

1）目的（Goal）

今現在、世界中を席巻している新型コロナウイルス（SARS-CoV-2）は、mRNAワクチンの標的であるスパイク遺伝子に次々と変異を起こすことにより、ワクチン接種者が獲得した中和抗体に対する耐性を獲得して、現在も収束することなく感染拡大を続けている。本研究において、第三の免疫「内因性免疫」を担うタンパク質としてヒト 細胞が有する抗ウイルス宿主因子のいくつかが、SARS-CoV-2に対しても有効か否かを検証し、その機能解析によって、本質的なウイルス感染防御に関する知見を得るとともに、将来的な感染再拡大に対する予防戦略を構築することを目的とする。

2）戦略（Approach）

ウイルスの複製に関わる宿主因子の研究が最先端レベルで進んでいるレトロウイルス学に本年度はまず着手して、分子ウイルス学的研究手法および宿主因子に関する知見について学ぶ。指導教官である徳永研三先生のチームが2015年に発見してNature Medicince誌（1）に報告した後に関連研究（2, 3, 4, 5）を次々と展開してきた抗ウイルス宿主因子MARCH8について、さらなる機能解析に取り組む。

3）材料と方法（Materials and methods）

i) 細胞：ヒト胎児腎細胞293T（6）をトランスフェクション用に、MAGIC5細胞（7）をウイルス感染用に使用した。サル（Rhesus macaque）、マウス、ウシMARCH8発現プラスミド作製用にRT-PCRの鋳型として必要な細胞RNAの抽出のために、それぞれアカゲザル網膜内皮細胞RF/6A（8）、マウス繊維芽細胞 NIH3T3（9）、ウシ腎臓細胞MDBK（10）を用いた。

ii) プラスミドDNA：シュードウイルス作製用にHIV-1エンベロープ糖タンパク質（Env）発現プラスミド pC-NLenv（1）、水胞性口炎ウイルスGタンパク質（VSV-G）発現プラスミド pC-VSVg（1）、HIV-1 Env欠損型ルシフェラーゼレポーターウイルスDNA pNL-Luc2-IN/HiBiT-E(-)Fin（11）を用いた。またヒトMARCH8発現プラスミドとしてpC-MARCH8（1）、RING-CH変異型MARCH8発現プラスミド pC-MARCH8-W114A（1）、チロシンモチーフ変異型MARCH8発現プラスミド pC-MARCH8-^{222}AxxL^{225}（3）を用いた。

iii) プラスミド構築：RF/6A、NIH3T3、およびMDBK細胞からReliaprep RNA Cell Miniprep system (Promega; Z6010)を用いて細胞RNAを抽出し、PrimeScript One Step RT-PCR Kit Ver. 2（Takara; RR057A） によりRT-PCR増幅を行った。得られたDNA断片を電気泳動後にアガロースゲルから切り出してQIAquick PCR Purification Kit（QIAGEN; 28104）を用いて精製した。さらに制限酵素 KpnI/XhoI で処理した最終断片を同じく KpnI/XhoI で処理した哺乳類細胞発現プラスミドpCAGGS（12）に挿入した。各々のRING-CH変異体およびチロシンモチーフ変異体を作製するため、3種類の動物由来の野生型MARCH8を鋳型に乗換えPCRを行い、増幅した KpnI/XhoI 断片をpCAGGSに挿入した。またこれら全てのN末HA-tag版も作製した。作製した全ての発現プラスミドはGenewiz遺伝子解析サービスにより遺伝子配列の確認を行った。

iv) トランスフェクション：pC-NLenvまたはpC-VSVg（20 ng）をpNL-Luc2-IN/HiBiT-E(-)Fin（500 ng）と各動物由来の野生型または変異型MARCH8発現プラスミド（0、30、60、120 ng）、さらに空プラスミドpCAGGS（480、450、420、360 ng）と共に、FuGENE6 Transfection Reagent（Promega; E2691）を用いて2.5×10^{5}個の293T細胞にコトランスフェクションした。

v) ウイルス定量：トランスフェクションの16時間後に293T細胞をPBSで洗浄して、更に24時間後に7.5 U/ml DNase I (Roche Applied Science; 11284932001)で処理した培養上清またはp24量が既知の標準ウイルス25 μLを、等量のHiBiT Lytic Substrate (1:50) in Nano-Glo HiBiT Lytic Buffer（Nano-Glo HiBiT Lytic Detection System; Promega; N3030）と混合して、10分間室温静置した後、Centro LB960 luminometer (Berthold)を用いてHiBiTルシフェラーゼ活性を測定した。

vi) 感染性アッセイ：各培養上清のHiBiTルシフェラーゼ活性をp24量に換算した後、1ng p24相当のウイルスを1x10^{4}個のMAGIC5細胞に感染させた。48時間後に各細胞を100 μLの One-Glo Luciferase Assay Reagent (Promega; E6110).で溶解して、感染性の指標となるホタルルシフェラーゼ活性を Centro LB960ルミノメーター(Berthold)によって測定した。

1. 研究概要(2)

3)材料と方法(Materials and methods) つづき

vii) ウエスタンブロッティング:各N末HA-tag付加MARCH8発現プラスミド(500 ng)と 空プラスミドpCAGGS(500 ng)を、FuGENE6を用いて2.5×10^5個の293T細胞にトランスフェクションした。48時間後に200 μLの細胞溶解液を加えてSDS-PAGEを行った後、PVDF膜に転写した。抗HA単クローン抗体 (Sigma; H9658)または抗β-actin単クローン抗体 (Sigma; A5316)を反応させ、Western ECL Substrate (Biorad; 1705061)で可視化した後、LAS-3000 imaging system (FujiFilm)で検出した。

4)実験結果(Results)

トランスフェクションおよびウエスタンブロッティング実験により、今回新たに作製したMARCH8発現プラスミドは全て同レベルで正常に発現していることが確認できた。動物種別MARCH8のHIV-1 EnvおよびVSV-Gに対する抑制効果について、感染性アッセイにより検討した結果、ヒトMARCH8と同様、サル、マウス、およびウシMARCH8の野生型はHIV-1 EnvとVSV-Gに対する量依存的な抑制能を保持していた。その一方でRING-CH変異型およびチロシンモチーフ変異型MARCH8はどちらも抑制活性を失っていた。

5)考察(Discussion)

MARCH8の抗ウイルスエンベロープ活性およびその機能領域は、異なる動物種間(ヒト、サル、マウス、およびウシ)で高度に保存されていることが明らかになった。

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						
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						
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掲載誌名 Published journal						
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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い

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

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					
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共同演者名 Co-presenter					
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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 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	
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

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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）　徳永　研三、上野　貴将