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 41 期　研究者番号：G4-03　作成日：2020 年 3 月 5 日

氏名	朱 俊	Zhu Jun	性別	M	生年月日 1980. 09. 18
所属機関（役職）	江蘇省蘇北人民医院（主治医師）				
研究先（指導教官）	順天堂大学大学院医学研究科眼科学（村上 晶教授）				
研究テーマ	骨髄由来免疫制御細胞のマウス角膜移植に及ぼす影響 Effect of ex-vivo induced myeloid-derived suppressor cells from bone marrow in a mouse corneal transplantation model				
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	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	角膜移植の治療において、血管侵入ある角膜混濁や再手術例では、免疫学的拒絶反応が今なお課題となっている。Myeloid-derived suppressor cells（MDSC）はリンパ節，末梢血に増加する未熟な骨髄細胞で強力な免疫抑制活性を示し、マウスではCD11b+Gr1+のMDSCが詳細に研究されている。このCD11b+Gr1+MDSCをex-vivo誘導し、角膜移植免疫反応のin vitroモデルに加えることで、T細胞の増殖抑制とTreg（制御性T細胞）の誘導が行われることを確認した。そのメカニズムにINF-γの産生の抑制とIL-2の産生増加が関連していることを観察しMDSCによる角膜移植免疫の制御治療の可能性を検討した。	
総合評価	【良かった点】 当初、眼科の外来診療と手術の研修に積極的に取り組まれた。本学眼科スタッフとの交流を行い、眼科全般の臨床経験が豊富な有能な臨床医であることを誰もが認めている。当初、網膜変性疾患の細胞移植治療研究を計画していたが、移植免疫の課題に興味をもち、本学で実験系が整備されていた角膜移植に関するプロジェクトに参画することになった。最新の免疫学、分子生物学について自己研修を行いながら、本学アトピーセンターの先生方の協力をいただき、教室の大学院生とともに研究を開始することができた。短期間で、実験動物の取り扱い、細胞培養の技術を習得し、熱心に研究取り組んでおり、高く評価できる。	
	【改善すべき点】特にないが、あえてあげれば、実験研究で時間の調整が大変だと思われるが、余裕があれば臨床のカンファレンスにまた是非参加してほしい。	
	【今後の展望】 研究の成果の一部はアジア太平洋眼科学会で報告する予定である。 拒絶反応をおこしやすい血管侵入のある角膜疾患モデルでの角膜移植における免疫制御への応用が可能かを検証し、論文作成をおこなう。	
学位取得見込	現在のペースで実験研究を行い、修了後に論文としてまとめることで十分レベルの研究成果を上げることは可能と思われます。	
評価者（指導教官名）	村上晶	

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41期　　研究者番号: G4103　　作成日:2020年3月 七 日

氏名	Zhu Jun	朱 俊	性別	M	生年月日 1980. 09. 18
所属機関(役職)	江蘇省蘇北人民医院(主治医師)				
研究先(指導教官)	順天堂大学大学院医学研究科眼科学(村上 晶教授)				
研究テーマ	骨髄由来免疫抑制細胞(MDSC)のマウス角膜移植に及ぼす影響 Effect of ex vivo-induced myeloid-derived suppressor cells from bone marrow in a mouse corneal transplantation model				
専攻種別	論文博士	☑	課程博士	☐	

1. 研究概要(1)

To investigate the effect of bone marrow (BM) derived myeloid-derived suppressor cells (BM-MDSCs) on T cell proliferative response on allogeneic stimulation in vitro.

1)目的(Goal)

To investigate the impact of bone marrow myeloid-derived suppressor cells (BM-MDSCs) on allogeneic stimulation in vitro and effect on the survival of mouse corneal transplantation in vivo.

2)戦略(Approach)

Corneal transplantation, as known as corneal grafting, is the most common surgery worldwide to treat various corneal diseases which cause severe vision loss. Although it is well-known that cornea has the immune privilege in the transplantation, there are still risk factors, such as neovascularization, inflammatory and infection, that could result in graft failure. It was reported that in some high-risk patients with inflamed and vascularized host beds, the graft failure could be 41% to 100%. [1-4] The immunologic rejection is considered as the main cause of corneal allotransplantation failure.[5] Medical therapy such as repeated surgery of corneal transplantation, pharmacotherapy, artificial cornea and bioengineered cornea provide diverse methods to improve survival of transplantation, however, the major therapy nowadays come from donor corneal transplantation, the main inevitable problem is to reduce the allograft immune rejection. Myeloid-derived suppressor cells (MDSCs) are heterogeneous population of myeloid cells. They were regarded as an important role to facilitate tumor progression by immune suppression. It was observed that MDSCs could suppress T cell-mediated immune response.[6] These features immediately arouse intense interests of their regulatory function in organ transplantation.[7,8] Few studies were reported about the effect of MDSCs in corneal transplantation and the suppressive mechanism remains unknown. Therefore, we investigate the function of MDSCs in allogeneic stimulation via vitro and their effect on mouse corneal allotransplantation survival.

3)材料と方法(Materials and methods)

BM cells were procured from C57BL/6J (B6) mouse and cultured with interleukin (IL)-6 and granulocyte macrophage-colony stimulating factor (GM-CSF) for 4 days to generate Gr1+CD11b+ BM-MDSCs. Mixed lymphocytes reaction (MLR) was performed by using BALB/c mouse lymphocytes, B6 mouse splenocytes (30Gy radiated) as simple allogeneic stimulation assay, and using B6 mouse BM-MDSCs to assess its effect on T cell proliferation and expression of inflammation cytokines. The T cell proliferation were assessed by thymidine uptake. The production of interferon-γ (IFN-γ) and IL-2 using enzyme-linked immunosorbent assay (ELISA). The ratio of regulatory T cells (Treg) in BALB/c mouse lymphocytes was investigated by using flow cytometric analysis after MLR. BALB/c mouse lymphocytes were labeled by using carboxyfluorescein diacetate succinimidyl ester (CFSE) to trace their proliferation in MLR.

4)実験結果(Results)

Co-cultured with GM-CSF and IL-6 significantly boosted the number of Gr1+CD11b+ BM-MDSCs compared with the control ($P < 0.05$). Comparing with simple allogeneic stimulation assay, BM-MDSCs significantly inhibited T cell proliferation and expanded the ratio of regulatory T cells ($P < 0.05$). BM-MDSCs significantly decreased the IFN-γproduction, whereas IL-2 production was increased ($P < 0.05$). CFSE-labeling assay showed that the BM-MDSCs significantly reduced the frequencies of CSFElow cell compared with BM-MDSCs absent assay ($P < 0.05$).

5)考察(Discussion)

1．研究概要(2)

BM-MDSCs have suppressive effect on inflammation response through reducing T cells proliferation and expanding Treg frequencies in vitro.This indicates BM-MDSCs are portential to promote survival on mouse corneal transplantation model. Further investigations are needed to interpret mechanism and prospective practice in corneal trasplantation.

6)参考文献(References)

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8.Gajardo T., Morales R.A., Campos-Mora M., et al. Exogenous interleukin-33 targets myeloid-derived suppressor cells and generates periphery-induced Foxp3(+) regulatory T cells in skin-transplanted mice. Immunology. 2015;146:81-88

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 lis

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

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

学会名 Conference	Asia-Pacific Academy of Ophthalmology Congress				
演　題 Topic	Endogenous Klebsiella Pneumoniae Endophthalmitis Originated From the Late-onset Liver Abscess				
開催日 date	2020　年　8　月　5　日	開催地 venue	Xiamen, China		
形式 method	☐ 口頭発表 Oral ☑ ポスター発表 Poste	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 Poste	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 Poste	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 Poste	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

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	2019/10/2
発表機関 Released medium	Subei Peoples' Hospital of Jiangsu Province		
発表形式 Release method	・新聞（WeChat Public Account of Subei Peoples' Hospital of Jiangsu Province）		
発表タイトル Released title	Doctors of Subei Feoples' Hospital of Jiangsu Provienc in abroad.		

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

Manuscript in submission:
Endogenous Klebsiella Pneumoniae Endophthalmitis Originated from Liver Abscess: Prognostic Barriers from the First Contact

指導責任者（署名） 村上昌司

今天，让我们一起倾听在海外、在他乡的他们对祖国的表白……

苏北人民医院 2019-10-02

微信扫一扫
关注该公众号

10月1日是中华人民共和国七十周年华诞。
七十年来，在中国共产党的领导下，
在中华儿女的共同努力下，
新中国取得了举世瞩目的成就。
在这样一个特别的日子里，
苏北人民医院有这样一群人
他们身在海外，
身在他乡，
更切身感受到了祖国的强大，
下面，
就让我们听一听他们对祖国母亲的深情告白……

08

70

眼科朱俊医生：
日本国顺天堂大学进修学习

中国，伟大的祖国！永远是海外学子最坚强的后盾！祝福祖国母亲生日快乐！

奋斗

是对祖国最好的表白

这是最好的祖国

还有更好的你

整理：海容 编辑：蒋潇潇

审核：汤　佳

文章已于2019-10-02修改

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 41 期　研究者番号：G4104　作成日：2020 年 3 月 5 日

氏名	孟　雪	Meng Xue	性別	F	生年月日 1986. 08. 23
所属機関（役職）	中国医科大学附属盛京医院（主治医師）				
研究先（指導教官）	順天堂大学大学院医学研究科耳鼻咽喉科学（池田 勝久主任教授）				
研究テーマ	次世代シークエンサーを用いた頭頸部癌の特異的癌遺伝子の創出 Detection of cancer-specific genes of head and neck carcinoma using next generation sequencer				
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	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	頭頸部扁平上皮癌の手術検体 302 例を用いて Tissue Micro Array (TMA)を作成。それらに対し免疫染色および FISH を行い、頭頸部癌に対する ACTN4 の機能解析を行っている。現在のところまでに TMA の作成が完成しており、現在免疫染色およぎ FISH の予備実験までが終了している。	
総合評価	【良かった点】 現在のところまで勤勉に研究・実験にとりくんでいる。 指導教官への報告も漏れなく定期的に行っている。	
	【改善すべき点】 現在のところ大きな問題はない	
	【今後の展望】 本研究を計画通り遂行する予定	
学位取得見込	来年度内には本研究の研究成果を学会および論文投稿までもっていけると考えている。奨学金支援内での学位取得に関しては不明だが、その後 3 年以内の取得は十分に可能と思われる。	
評価者（指導教官名）　松本文彦　(印)		

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41期　　研究者番号: G4104　　作成日:2020年3月 4 日

氏名	Meng Xue	孟 雪	性別	F	生年月日 1986. 08. 23
所属機関(役職)	中国医科大学附属盛京医院(主治医師)				
研究先(指導教官)	順天堂大学大学院医学研究科耳鼻咽喉科学(池田 勝久主任教授)				
研究テーマ	次世代シークエンサーを用いた頭頸部癌の特異的癌遺伝子の創出 Detection of cancer-specific genes of head and neck carcinoma using next generation sequencer				
専攻種別	論文博士	☑	課程博士	☐	

1. 研究概要(1)

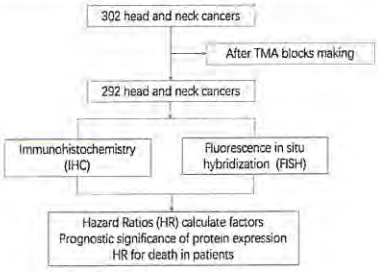
Head and neck squamous carcinoma (HNSCC) is the six most common type of cancer worldwide, accounts for approximately 550,000 new cases and approximately 300,000 deaths each year worldwide. The 5-year overall survival rate remains unfavorable, so the poor prognosis of patients with HNSCC highlights the need to elucidate the new prognostic biomarker of HNSCC. A retrospective study was performed to demonstrate the potential usefulness prognostic biomarker of ACTN4 in patients with HNSCC.

1)目的(Goal)

A retrospective study was performed to demonstrate the potential usefulness prognostic biomarker of ACTN4 gene amplification and IHC expression in patients with HNSCC.

2)戦略(Approach)

Please see Fig. 1 which shows the approach in my study.



3)材料と方法(Materials and methods)

Patients and tissue sample

We reviewed the clinicopathological records of 292 patients who underwent surgical resection with curative intention for head and neck cancer at the National Cancer Center Central Hospital (Tokyo, Japan) between 2006 and 2016.

Formalin-fixed paraffin-embedded tissue samples of 292 head and neck cancers were enrolled retrospectively in this study. The study was performed in accordance with the reporting recommendations for tumour marker prognostic studies (REMARK) guidelines (Table. 1)[1,2]. None of the patients had undergone adjuvant chemotherapy. This study was approved by the ethics committee of the National Cancer Center.

TMA construction

Tissue microarrays (TMAs) were prepared from formalin- fixed paraffin-embedded pathological blocks, as previously described[3]. TMA blocks were cut into 4-lm-thick sections, the core diameter is 2.0 mm and subjected to FISH and immunohistochemistry (IHC).

Immunohistochemistry (IHC)

The anti-actinin-4 monoclonal antibody (13G9), which was originally established by the present study group, was purchased from Abnova Inc. (Taipei, Taiwan)[4]. Actinin-4 was immunostained using the Ventana DABMap Detection Kit and automated slide stainer (Discovery XT) (Ventana Medical Systems Inc., Tucson, AZ, USA)[5,6].

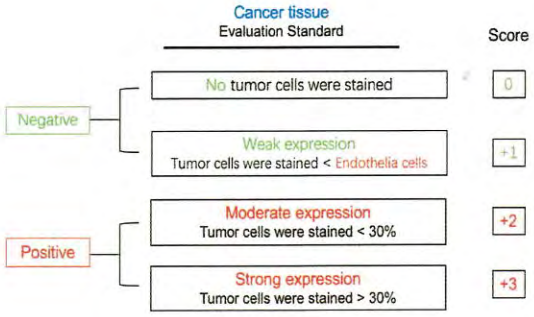
The expression level of actinin-4 protein was classified as: no expression (immunoreactivity score, 0), in which no tumor cells were stained with anti-actinin-4 antibody; weak expression (+1), in which tumor cells were stained with weaker intensity than endothelial cells; moderate expression (+2), in which less than 30% of tumor cells were stained; and strong expression (+3), in which more than 30% of tumor cells were stained. The negative group (actinin-4-negative) was defined as those cases that did not show actinin-4-positive staining, as described previously[7,8,9]. Two independent investigators who had no clinical information about the cases evaluated the staining patterns.

Fluorescence in situ hybridization

The FISH probe of bacterial artificial chromosome clone containing ACTN4 and chromosome 19p (CEP19; a control clone) were purchased from Abnova (Taipei, Taiwan)[10]. The labeled bacterial artificial chromosome clone DNA was subjected to FISH as previously described. TMAs were hybridized with FISH probes at 37° C for 48 h. The nuclei were counterstained with 4, 6-duamidi- no-2-phenylindone. The number of fluorescence signals corresponding to the copy number of ACTN4 and control signals in the nuclei of 20 interphase tumor cells was counted.

FISH patterns were categorized as described previously[11,12,13]. Briefly, the samples were grouped as 'gene amplification' (i.e., FISH-positive) if the ACTN4/CEP19 ratio was >2.0, or 'no gene amplification' (i.e., FISH-negative) if the ACTN4/CEP19 ratio was <2.0, as per the US Food and Drug Administration recommendations for HER2 tests (human epidermal growth factor receptor 2).

1．研究概要(2)
4)実験結果(Results)
1. ACTN4 IHC Evaluation in cancer tissues
Baseline characteristics of the patients with positive/negative IHC staining for actinin-4 was showed in Table 1 below.



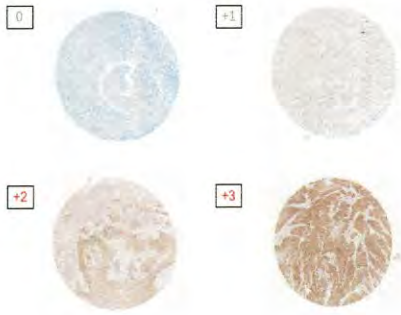
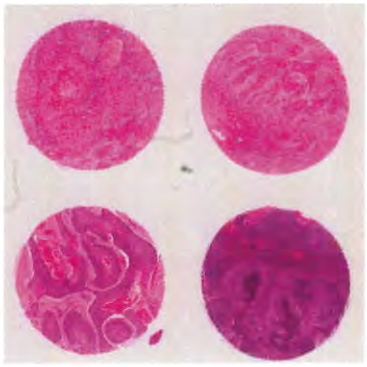


Table. 1 Baseline characteristics of the patients with positive/negative IHC staining for actinin-4

	Number of cases (%)		ACTN4 Positive (+2, +3)	ACTN4 Negative (0, +1)	*P*-value
Total	292		248	44	
Disease					
Oral cancer	152	52.70%	132	20	
Tongue cancer	82	53.2%	76	6	
Mouth Floor cancer	18	11.7%	16	2	
Pharygeal cancer	93	31.8%	81	12	
Laryngeal cancer	22	7.5%	13	0	
Esophageal cancer	17	5.8%	16	1	
Others	8	2.7%	7	1	
Age, years					0.421
Median(range)	67.53±11.12	(29-95)			
≤60	71	24.3%	64	7	
>60	221	75.70%	184	37	
Sex					0.249
Male	226	77.40%	189	37	
Female	66	22.60%	59	7	



2. ACTN4 IHC Evaluation in non-cancer tissues
Baseline case-control characteristics in non-cancers was showed in Table 2 below.

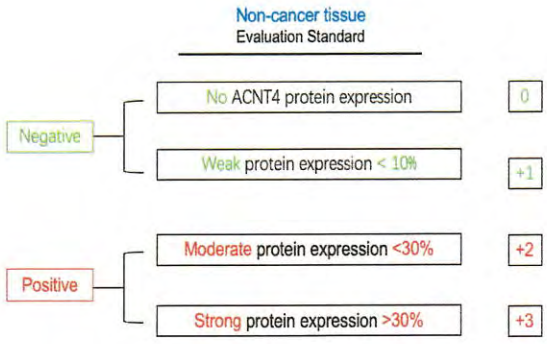
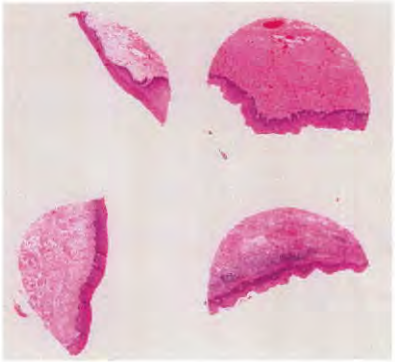


Table.2 Baseline case-control characteristics in non-cancers

	Number of cases (%)	
Total	99	
Age, years		
Median(range)	67.38±11.18	(29-95)
≤60	21	24.3%
>60	78	75.70%
Sex		
Male	72	77.40%
Female	27	22.60%



1. 研究概要(3)
5)考察(Discussion)
The gene ACTN4 was identified by the present researchers as cancer-associated. In our laboratory, we identified the ACTN4 gene product as an actin-bundling protein that was closely associated with cell movement and cancer invasion [14].
In some studies, actinin-4 overexpression was also confirmed in malignant tumors, and protein expression was a bad predictor of patient outcome. Actinin-4 is essential for the cellular processes that are associated with cell motility and cancer invasion, and overexpression of the actinin-4 protein switches the cancer cell to invasive. The cases of actinin-4 protein overexpression have been reported in a number of cancers, we have found in our lab. They are breast cancer, colorectal cancer, pancreatic cancer, bladder cancer, thyroid cancer, NSCLC, and salivary gland carcinoma.
The present studies, I successfully finished IHC staining, and analysis the data recently. The study data suggest that the outcome of expression of ACTN4 protein in TMA block maybe a potential biomarker, because of the hight ACTN4 protein expression. Next step is FISH in ACTN4. Depending on the result of IHC and FISH, we can continue analysis the other clinical data.

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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	The Effects of HBXIP on the Biological Functions of TSCCa Cells and correlation with PI3K/Akt					
掲載誌名 Published journal	Translational Cancer Research (2020.03.03 accept)					
	2020 年 3 月	巻(号)	頁 ～ 頁	言語 Language	English	
第1著者名 First author	Xue Meng	第2著者名 Second author		第3著者名 Third author		
その他著者名 Other authors	Corresponding author: Weixian Liu					
論文名 2 Title	Mandibular Malignant Fibrous Histiocytoma:a Case Report					
掲載誌名 Published journal	Journal of China Medical University					
	2019 年 9 月	48(9) 巻(号)	859 頁 ～ 861 頁	言語 Language	Chinese	
第1著者名 First author	Ye Li	第2著者名 Second author	Haiyang Yu	第3著者名 Third author	Qiuxu Wang	
その他著者名 Other authors	Corresponding author: Xue Meng					
論文名 3 Title	LncRNA DANCR promotes the proliferation, migration, and invasion of tongue squamous cell carcinoma cells through miR-135a-5p/KLF8 axis					
掲載誌名 Published journal	Cancer Cell Int					
	2019 年 11 月	19 巻(号)	302 頁 ～ 316 頁	言語 Language	English	
第1著者名 First author	Zheng Y	第2著者名 Second author	Zheng B	第3著者名 Third author	Meng X	
その他著者名 Other authors	Yan Y, He J, Corresponding author: Liu Y.					
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	15th Japan-Taiwan Conference on Otolaryngology-Head and Neck Surgery				
演 題 Topic	The effects of HBXIP on the Biological Functions of TSCCa Cell Line and the correlation with PI3K/Akt				
開催日 date	2019 年 12 月 6 日	開催地 venue	福岡		
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	Katsuhisa Ikeda, Fumihiko Matsumoto, Weixian Liu				
学会名 Conference	第38回日本耳鼻咽喉科免疫アレルギー学会総会・学術講演会				
演 題 Topic	The effects of HBXIP on the Biological Functions of TSCCa Cell Line and the correlation with PI3K/Akt				
開催日 date	2020 年 2 月 28 日	開催地 venue	横浜(延期)		
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	Katsuhisa Ikeda, Fumihiko Matsumoto, Weixian Liu, Kazufumi Honda				
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	公益財団法人国際耳鼻喉科学振興会
助成金名称 Grant name	Society for Promotion of International Oto-Rhino-Laryngology(SPIO)
受給期間 Supported period	2020 年 4 月 ～ 2021 年 3 月
受給額 Amount received	2,500,000 円
受給実績 Receipt record	□ 有　□ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円

6. 他の奨学金受給 Another awarded scholarship

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

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

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

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

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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（署名）　池田勝久　㊞

·短篇论著·

下颌骨恶性纤维组织细胞瘤1例报告

Mandibular Malignant Fibrous Histiocytoma:a Case Report

李野[1],于海洋[2],王秋旭[2],刘维贤[2],孟雪[2]
(1.北京大学深圳医院口腔科,广东 深圳 518046;2.中国医科大学附属盛京医院口腔颌面外科,沈阳 110004)

摘要 恶性纤维组织细胞瘤(MFH)是老年人最常见的恶性肿瘤之一。MFH常见于四肢软组织和腹膜后,位于下颌骨者极为罕见。手术切除是MFH患者的首选治疗方法,放疗和化疗可能有效。本文报告1例下颌骨MFH,并结合相关文献进行分析。

关键词 恶性纤维组织细胞瘤;颌骨;罕见肉瘤
中图分类号 R782 **文献标志码** A **文章编号** 0258-4646(2019)09-0859-03
网络出版地址 http://kns.cnki.net/kcms/detail/21.1227.R.20190906.1316.044.html
DOI:10.12007/j.issn.0258-4646.2019.09.022

纤维组织细胞瘤是细胞分化成纤维细胞和组织细胞而形成的肿瘤。其中只有一小部分表现为恶性,称为恶性纤维组织细胞瘤(malignant fibrous histiocytoma,MFH)[1],也称为未分化多形性肉瘤,是最常见的成人软组织肉瘤[2],由多形性纺锤和上皮样细胞组成,有少量多核细胞[3]。MFH可发生于广泛的年龄范围,常见于50~70岁年龄组,男性多见,在少数情况下,MFH发生于儿童,但侵略性较差。MFH好发部位为四肢软组织和腹膜后[4],发生在头部和颈部相对较少,发病率约3%~10%,可影响鼻腔、颅骨、喉部和颈部的软组织[5]。研究[6]表明,头颈部最常见的部位是上颌窦(5/15)、颈(4/15)和颞下窝(2/15),发生于下颌骨的MHF比较少见,仅占所有MFH骨病变的3%[7-8]。现回顾中国医科大学附属盛京医院口腔颌面外科收治的1例下颌骨MFH患者的诊治经过,总结其治疗经验和体会。

1 临床资料

患者,女,62岁,2016年9月因头疼就医。完善颈椎CT时发现右下后牙区破坏。数日后颈部淋巴结肿大,口服抗生素,症状缓解。同年10月,右下唇皮肤有麻木感,右下后牙咬合痛。入院检查口内可见右下第一磨牙根方舌侧黏膜隆起约0.5 cm × 0.5 cm,表面光滑,质韧,界限清楚,无触压痛。颈部未触及肿大淋巴结。无全身系统性疾病。CT显示右下颌骨局部略膨胀,内见软组织密度影,边界欠清,冠状面长径约33 mm,骨皮质膨胀变薄、局部显示不清,提示右下颌骨占位性病变,见图1。

完善术前检查后,患者在全身麻醉下行下颌骨部分切除术+自体骨移植术(右髂前上棘)+下颌骨缺损钛板坚固内固定术。术后常规抗炎、换药及护理治疗,术区及供区愈合良好并顺利出院。术后45 d术区出现感染症状,反复肿胀和疼痛,皮肤出现瘘道,给予换药,明显好转。术后2个月,因意外的外力打击导致钛板折断,但未出现明显移位,且植入区断端可见明显新生骨质形成,继续给予局部冲洗、换药及抗生素治疗,半年后,感染完全消失,窦道愈合。术后随访1年,未再次出现肿胀及疼痛等相关感染症状,骨折断端愈合良好。见图2。

病理检查显示,光镜下可见瘤组织,由呈束状、密集排列的梭形瘤细胞构成,局部变性,未见坏死,每10个高倍视野约有26个分裂细胞。免疫组化:Vimentin(+);CD68(+);Ki-67(约20%+);SMA(-);Desmin(-);CD34(血管-);S-100(-)。见图3。

2 讨论

MFH是一种最常见的软组织肉瘤[9]。MFH通常分为多形性、黏液性、巨细胞、血管瘤性和炎症亚型5种组织学类型,预后较好的为黏液性及血管瘤性,而与巨细胞变异相关的预后较差[10]。在1个病灶

作者简介:李野(1992-),女,医师,硕士.
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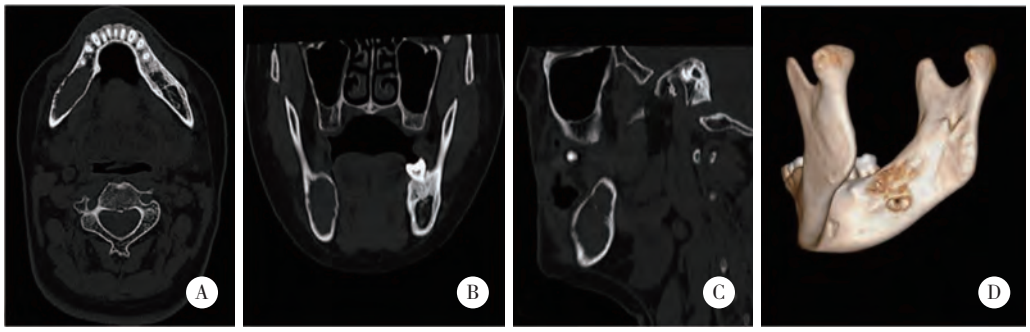
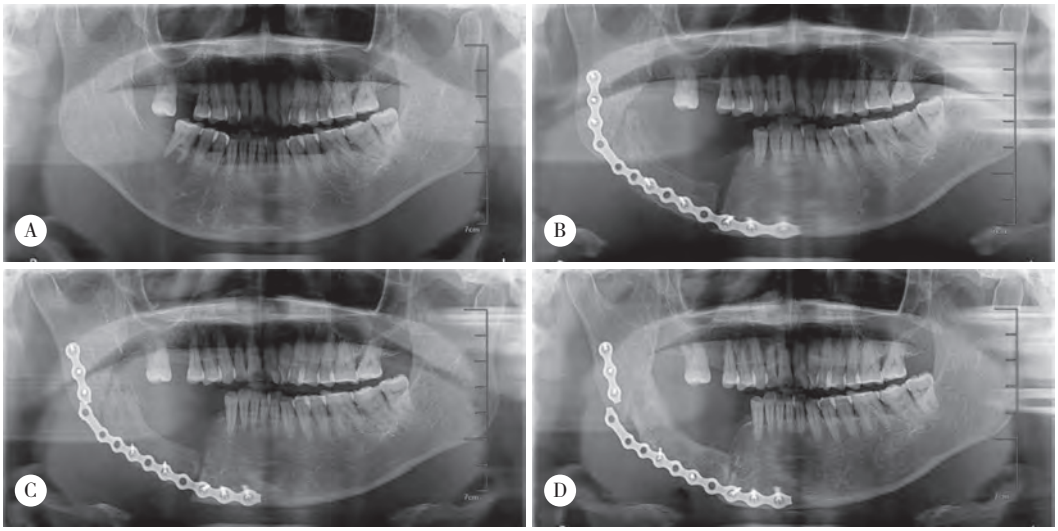


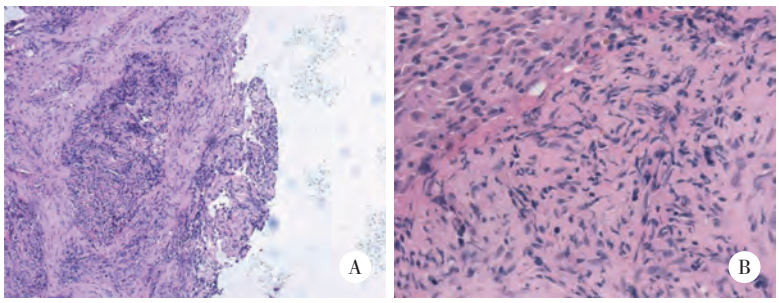
A,横断位;B,冠状位;C,矢状位;D,三维重建CT.

图1　CT检查结果



A,术前;B,术后1个月;C,术后2个月,患者受外力打击致钛板折断;D,术后1年,植骨断端可见明显新生骨质形成.

图2　术前及术后CT结果



A,×10;B,×40.

图3　病理光镜下HE染色结果

内常可见到几种组织学类型同时存在,这可能与细胞分化优势有关[11]。尽管MFH诊断率很高,但仍然未确定其真正的起源细胞[8]。MFH的发病原因目前也尚未完全确定,据报道,MFH是头部和颈部区域最常见的辐射诱发肉瘤,CAI等[12]描述了59例头部和颈部的辐射诱发肉瘤,其中包括10例(16.9%)MFH。也有学者认为MFH是放射治疗、慢性术后修复、创伤、手术切口或烧伤疤痕后的并发症。此外,MFH与血液系统疾病有关,如非霍奇金淋巴瘤、霍奇金淋巴瘤、多发性骨髓瘤和恶性组织细胞增生症,大约20%的病例有创伤史。颌骨MFH最常见的症状是肿胀,疼痛,出血,伴分泌物[8]。MFH影像学无特异性表现,软组织侵犯和骨组织破坏比较常见,在头颈部的MFH中,可能会出现明显而长期的

增强信号。如果患者有放射治疗史，则应考虑MFH的诊断[13]。MFH没有标准的治疗指南。手术切除肿瘤是主要的治疗方法，所有接受手术切除的患者5年生存率为67.2%[14]，未切除MFH患者的5年生存率<10%[15]。对于远处转移的高级别肿瘤，结合放射治疗和辅助化疗在内的多模式治疗可能会取得更好的结果[16-17]。晚期MFH的标准治疗方法是化疗，主要以阿霉素和异环磷酰胺作为一线治疗药物，单独或联合治疗[18]。MFH联合化疗的药物毒性常常导致治疗停止，尤其常见于老年患者[19]。另外，阿帕替尼可能为MFH的治疗提供了一个额外的选择[20]。MFH可通过血行播散，主要发生在肺部（82%），转移和复发的决定因素是组织学和肿瘤大小[21]。非黏液性MFH较黏液性MFH的转移倾向高，非黏液样病变超过5.0 cm的患者有出现转移的危险[22]。综上所述，手术治疗以及放化疗需要考虑到患者的年龄、并发症及肿瘤组织分型。如果肿瘤化学敏感，毗邻重要器官，术前可化疗[23]。不同类型的MFH化学敏感性和预后不同，MFH的风险分层对于治疗适应证尤其重要[24]。

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LncRNA DANCR promotes the proliferation, migration, and invasion of tongue squamous cell carcinoma cells through miR-135a-5p/KLF8 axis

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Abstract

Background: Tongue squamous cell carcinoma (TSCC) is a most invasive cancer with high mortality and poor prognosis. It is reported that lncRNA DANCR has implications in multiple types of cancers. However, its biological role and underlying mechanism in TSCC progress are not well elucidated.

Methods: Our present study first investigated the function of DANCR on the proliferation, migration and invasion of TSCC cells by silencing or overexpressing DANCR. Further, the miR-135a-5p-Kruppel-like Factor 8 (KLF8) axis was focused on to explore the regulatory mechanism of DANCR on TSCC cell malignant phenotypes. Xenografted tumor growth using nude mice was performed to examine the role of DANCR in vivo.

Results: DANCR knockdown reduced the viability and inhibited the migration and invasion of TSCC cells in vitro, while ectopic expression of DANCR induced opposite effects. In vivo, the tumor growth and the expression of matrix metalloproteinase (MMP)-2/9 and KLF8 were also blocked by DANCR inhibition. In addition, we found that miR-135-5p directly targeted DANCR, which was negatively correlated with DANCR on TSCC progression. Its inhibition reversed the beneficial effects of DANCR silence on TSCC malignancies. Furthermore, the expression of KLF8 evidently altered by both DANCR and miR-135a-5p. Silencing KLF8 using its specific siRNA showed that KLF8 was responsible for the induction of miR-135a-5p inhibitor on TSCC cell malignancies and MMP-2/9 expression.

Conclusions: These findings, for the first time, suggest that DANCR plays an oncogenic role in TSCC progression via targeting miR-135a-5p/KLF8 axis, which provides a promising biomarker and treatment approach for preventing TSCC.

Keywords: DANCR, Tongue squamous cell carcinoma, miR-135a-5p, KLF8, MMP

Background

Tongue squamous cell carcinoma (TSCC) is a major type of head and neck squamous cell carcinoma (HNSCC) with high recurrence rates, increased proliferation and metastasis, and poor prognosis [1, 2]. Despite of significant advances in the prevention and treatment, the survival rates of TSCC patients are still low [3]. It is identified that the invasion and migration mainly contribute to the progression of tumors. Therefore, it is urgent that developing novel therapeutic strategies for TSCC through the exploration of the underlying molecular mechanisms.

LncRNAs are a group of long non-coding RNAs with more than 200 nucleotides in length. Numerous reports has shown that lncRNAs play important roles in wide ranges of biological processes, including cell proliferation, differentiation, apoptosis, migration and invasion [4–6]. Especially, multiple lncRNAs has been found to be closely implicated in the tumorigenesis and progression of TSCC. For example, high-expression of lncRNA

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AFAP1-AS1 in TSCC tumor tissues enhances tumor progression via the activation of Wnt/β-catenin signaling pathway [7]. NKILA serves as a crucial determinant of TSCC metastasis to reduce the migratory and invasive cells through inhibiting the process of epithelial–mesenchymal transition (EMT) [8]. Interestingly, lncRNA DANCR (differentiation antagonizing non-protein coding RNA) has been noticed to suppress epidermal cell differentiation [9] and improve hepatocellular carcinoma self-renewal [10]. DANCR is also taken as an oncogenic lncRNA for several cancers, such as prostate cancer [11], gastric cancer [12] and colorectal cancer [13]. However, the distinct function of DANCR in TSCC was not well understood.

MicroRNAs (miRNAs), a class of small non-coding RNAs, are shown to modulate the expression of target genes. Recent studies have revealed that miR-135a-5p is the main regulator of tumor invasion and metastasis [14, 15]. In non-small cell lung cancer (NSCLC), miR-135a-5p is demonstrated to inhibit cell migration and invasion through targeting Kruppel-like Factor 8 (KLF8) [16]. As we know, KLF8 has been widely confirmed to participate in the regulation of cell cycle progression, transformation, EMT and invasion [17–21]. Given that DANCR was predicted to have putative binding sites with miR-135a-5p through the analysis of online bioinformatics, we thus speculated that DANCR might affect the development and progression of TSCC by regulating miR-135a-5p/KLF8 axis.

To improve the understanding of DANCR effects on TSCC malignancies, CAL-27 and TCa-8113 cells with DANCR silence, and SCC9 and TSCCA cells with DANCR overexpression were constructed. Then the effects of DANCR on the proliferation, migration and invasion of TSCC cells were determined. Further, miR-135a-5p/KLF8 axis was focused to explore the molecular mechanism by which DANCR promoted TSCC progression.

Methods

Cell culture and reagents

In our experiments, four human TSCC cell lines (SCC9, TSCCA, TCa-8113 and CAL-27 cells) were used. SCC9 cells (Cellcook, Guangzhou, China) were cultured in DMEM/F12 medium supplemented with 10% fetal bovine serum (FBS; SH30084.03, Hyclone, South Logan, UT, USA); TSCCA cells (Procell, Wuhan, China) were maintained in DMEM medium (12100-46, Gibco) containing with 10% FBS; TCa-8113 and CAL-27 cell lines (Procell, Wuhan, China) were cultured in RPMI-1640 medium (31800-014, Gibco, Gaithersburg, MD, USA) supplemented with 10% FBS. All these cell lines were cultured in a standard environment at 37 °C with 5% CO_2. MiR-135a-5p mimics/inhibitor and corresponding negative control (NC) mimics/inhibitor were purchased from JTS Scientific (Beijing, China).

Construction of siRNAs and shRNAs

The sequences of siRNAs (5′–3′) targeting human DANCR were designed as follows: si-DANCR-1 sense GUUGACAACUACAGGCACATT and antisense UGU GCCUGUAGUUGUCAACTT; si-DANCR-2 sense CUA GAGCAGUGACAAUGCUTT and antisense AGCAUU GUCACUGCUCUAGTT. The NC siRNA sequences (5′–3′) were: sense UUCUCCGAACGUGUCACGUTT and antisense ACGUGACACGUUCGGAGAATT. Then shRNAs targeting DANCR and corresponding NC were constructed by pRNAH1.1 plasmid vectors (Genscript, Nanjing, China).

Furthermore, we also designed the interfering sequences (5′–3′) for human KLF8 as follows: si-KLF8 sense CGAUAUGGAUAAACUCAUATT and antisense UAUGAGUUUAUCCAUAUCGAC. The corresponding NC siRNA sequences (5′–3′) were designed as follows: si-NC sense UUCUCCGAACGUGUCACGUTT and antisense ACGUGACACGUUCGGAGAATT.

Construction of overexpression plasmids

A pair of specific primers (forward 5′-CAAGGATCC GCCCTTGCCCAGAGTCTTCC-3′ and reverse 5′-CCG CTCGAGGTCAGGCCAAGTAAGTTTAT-3′) was used to amplify human DANCR (NR_024031.2). Then the amplified products were inserted into pcDNA3.1 plasmids (V790-20, Invitrogen, Carlsbad, CA, USA) between BamHI and XhoI restriction enzyme sites to induce the overexpression of DANCR. The empty pcDNA3.1 vector was used as control.

Cell transfection

When cells reached at 70% of confluence, siRNAs or shRNAs targeting DANCR were transfected into CAL-27 and TCa-8113 cells, and ectopic expression of DANCR were transfected into SCC9 and TSCCA cells by the mediation of Lipofectamine 2000 reagent (11668-019, Invitrogen) following the manufacturer's instructions. All experiments were performed at 48 h post transfection.

In addition, miR-135a-5p mimics or NC mimics was transfected into CAL-27 or TCa-8113 cells, and its inhibitor or NC inhibitor was transfected into SCC9 cells as mentioned above to overexpress or silence miR-135a-5p. Furthermore, the co-transfection of miR-135a-5p inhibitor and si-DANCR or si-KLF8 was also mediated by Lipofectamine 2000.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNAs in TSCC cell lines were extracted with RNAsimple Total RNA Kit (DP419, TIANGEN, Beijing, China) and reverse-transcribed into cDNA templates using M-MLV reverse transcriptase (NG212, TIANGEN). The designed specific primer sequences were synthesized by Sangon Biotech (Shanghai, China) and shown as follows (5′–3′): miR-135a-5p, RT GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACTCACAT, forward GCCGTATGGCTTTTTATTCCTA and reverse GGTGCAGGGTCCGAGGTATT; U6, RT GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACAAAATATGG, forward GCTTCGGCAGCACATATACT and reverse GGTGCAGGGTCCGAGGTATT; DANCR forward ACCCTCCTGCTTCCCTC and reverse CCCGAAACCCGCTACAT; KLF8 forward TCATTGGAGGAGATGGTAA and reverse GCTGCTGGTTCTTGCTGT; GAPDH forward GACCTGACCTGCCGTCTAG and reverse AGGAGTGGGTGTCGCTGT. Subsequently, the mixture of cDNA templates, specific primers, SYBR Green reagent (SY1020, Solarbio, Beijing, China) and Taq PCR MasterMix (KT201, TIANGEN) were used to amplify target genes by qRT-PCR analysis on Exicycler 96 PCR system (Bioneer, Daejeon, Korea). GAPDH was normalized for DANCR and KLF8 expression, and U6 was normalized for miR-135a-5p expression. Relative expression was calculated using the $2^{-\Delta\Delta CT}$ method.

MTT assay

TSCC cells were seeded in 96-well plates at the density of 4×10^3 cells/well for 0, 24, 48 or 72 h, respectively. Then cells were incubated in a complete medium containing 0.5 mg/ml MTT (KGA311, KeyGEN, Nanjing, China) for 4 h. After dissolving in DMSO (ST038, Beyotime), the viable cells were determined using microplate reader (ELX-800, BIOTEK, Winooski, VT, USA) at the optical density of 570 nm.

Wound healing assay

The wound healing assay was used to assess cell migratory ability. Cells were treated with mitomycin C (M0503, Sigma) for 1 h in a serum-free medium. Then a wound scratch was made by a 200 μl pipette tip in the culture plate and recorded it by phase-contrast microscopy (IX53, Olympus, Tokyo, Japan) under 100× magnification. Twenty-four hours later, the migratory distances were measured with Image Pro Plus Software (Media Cybernetics, Silver Springs, MD, USA) to calculate the capacity of cell migration.

Transwell assay

Transwell assay was utilized to evaluate the invasive ability of cells. Briefly, cell suspensions (2×10^4 cells/well) were seeded in the upper chamber of 24-well Transwell inserts (3422, Corning Incorporated, Corning, NY, USA) pre-coated with Matrigel (356234, BD Biosciences, San Jose, CA, USA) with serum-free medium. The lower chamber was filled with the medium containing with 30% FBS. After 48 h of incubation, cells in the upper chamber were removed and washed in PBS twice. Then cells were fixed in 4% paraformaldehyde and stained with 0.4% crystal violet (0528, Amresco, Solon, OH, USA). The number of cells in the lower chamber was observed by phase-contrast microscope under 200× magnification. Five fields in each image were randomly selected to count and the invasive cell ratio was normalized to control.

Luciferase reporter assay

Bioinformatics analysis predicted that lncRNA DANCR had putative binding sites with miR-135a-5p. The pmirGLO vector (E133A, Promega, Madison, WI, USA) containing NheI and SalI restriction enzyme sites was applied to construct wild type (wt) or mutant type (mut) luciferase reporter vectors for DANCR. The site-directed mutation of DANCR was used to verify the target effects between DANCR and miR-135a-5p. Then 293T cells (ZhongQiaoXinZhou Bio, Shanghai, China) were seeded in 12-well plates and co-transfected with wt-DANCR, or mut-DANCR together with miR-135a-5p or NC mimics using Lipofectamine 2000. Finally, the binding activity was tested with a dual luciferase reporter assay kit (E1910, Promega) by the calculation of Firefly luciferase activity/Renilla luciferase activity at 48 h post-transfection.

Western blot

Total proteins from TSCC cell lines or tumor tissues were isolated using RIPA lysate (R0010, Solarbio) containing PMSF (P0100, Solarbio) and quantified using BCA assay kit (PC0020, Solarbio). Then equal proteins were loaded on the Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) gel, and transferred onto PVDF membrane (IPVH00010, Millipore, Billerica, MA, USA). After washing in TBST, the membrane was incubated with one of the following specific primary antibodies overnight at 4 °C: MMP-2 antibody (1:500; 10373-2-AP, Proteintech, Wuhan, China), MMP-9 antibody (1:500; ab38898, Abcam, Cambridge, UK), KLF8 antibody (1:1000; A16321, Abclonal, Wuhan, China) and GAPDH (1:10,000; 60004-1-Ig, Proteintech). Subsequently, horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody (1:3000; SE134, Solarbio) or HRP-conjugated goat anti-mouse antibody (1:3000; SE131, Solarbio) was

used to incubate with the membrane for 1 h at 37 °C. Protein signals were developed with ECL kit (PE0010, Solarbio) and quantified using Gel-Pro-Analyzer Software (Media Cybernetics, Silver Springs, MD, USA). GAPDH was used as internal control.

Xenograft tumor model analysis

The ethical approval was obtained from School of Stomatology, China Medical University Committee (No. G2018007) in this study. All animal experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals. The Balb/c-nude mice (4–5 weeks, 18–20 g) were purchased from HuaFu-Kang Bioscience Co. lnc (Beijing, China) and housed in a standard environment. Stably transfected cells with sh-DANCR or sh-NC were selected using G418 antibiotics (A1720, Sigma, St. Louis, MO, USA). Then, CAL-27 cells or TCa-8113 cells with sh-DANCR or sh-NC stable transfections were subcutaneously injected into the right side of axilla at the density of 1×10^6 cells per animal. Tumor volume was measured using the caliper every 4 days following the formula: tumor volume (mm^3) = (length × width^2)/2. Tumor weight was measured when mice were killed after 25 days.

Immunofluorescence

For immunofluorescence staining, the collected tumor tissues were fixed in paraformaldehyde, embedded with paraffin and sectioned into 5 μm-thickness slides. Then paraffin slides were incubated with specific primary antibody against KLF8 (NBP2-57740, NOVUS, Centennial, CO, USA) overnight at 4 °C, and conjugated with FITC-labeled goat anti-rabbit secondary antibody (A0562, Beyotime) at room temperature for 60 min. After counterstaining with DAPI, the immunopositive materials were visualized using optical microscope (BX53, Olympus) at the magnification of 400× and captured using digital camera (DP73, Olympus).

Statistical analysis

Data were expressed as mean ± SD and analyzed using GraphPad Prism software (San Diego, CA, USA). The comparisons were performed using t-test or one-way ANOVA following Bonferroni's test. $p < 0.05$ was identified to indicate a significant difference statistically.

Results

DANCR knockdown suppressed the proliferation, migration and invasion of TSCC cell lines

In four different TSCC cell lines, the expression profile of DANCR was first detected as shown in Fig. 1a. From this chart, it was apparent that DANCR expression was higher in CAL-27 and TCa-8113 cells than in SCC9 and TSCCA cells. Thus in further experiments, CAL-27 and TCa-8113 cells were used to inhibit DANCR, while SCC9 and TSCCA cells were forced to express DANCR. As expectation, specific siRNAs targeting DANCR significantly decreased its levels in CAL-27 and TCa-8113 cells (Fig. 1b).

Then the effects of si-DANCRs on the proliferation, migration and invasion of TSCC cells were first assessed. MTT assay was considered to indicate cell proliferation, and the results showed that DANCR knockdown reduced the viable number of CAL-27 and TCa-8113 cells (Fig. 1c). Furthermore, it seemed that inhibition of DANCR significantly decreased the migratory and invasive ability of TSCC cells using wound healing assay and transwell invasion assay (Fig. 1d, e). These results indicate that DANCR knockdown may attenuate TSCC malignancies in vitro.

DANCR overexpression promoted the proliferation, migration and invasion of TSCC cell lines

Further, the forced expression of DANCR was used to investigate its biological function in SCC9 and TSCCA cells. We observed a marked increase of DANCR expression by its overexpression plasmids in SCC9 and TSCCA cells (Fig. 2a). Functional analysis from SCC9 and TSCCA cells indicated that the ectopic expression of DANCR induced increments of cell viability, migratory distance and invasive cell number (Fig. 2b–d). Our data show that DANCR can enhance the proliferation, migration and invasion of TSCC cells in vitro.

DANCR targeted miR-135a-5p to regulate KLF8 expression in TSCC cell lines

As shown in Fig. 3a, the bioinformatics predicted that DANCR was complementary with miR-135a-5p (Fig. 3a), which was confirmed by dual luciferase reporter assay. The results demonstrated that miR-135a-5p mimics significantly inhibited the luciferase activity of wt-DANCR, but not mut-DANCR (Fig. 3b). Then we observed a marked increase of miR-135a-5p level in CAL-27 and TCa-8113 cells transfected with si-DANCR (Fig. 3c, d), but a significant reduction of miR-135a-5p in SCC9 and TSCCA cells transfected with pcDNA3.1-DANCR (Fig. 3e, f). In addition, KLF8 mRNA was down-expressed by knockdown of DANCR in CAL-27 (Fig. 3g) and TCa-8113 cells (Fig. 3h), but increased by DANCR overexpression in SCC9 (Fig. 3i) and TSCCA cells (Fig. 3j). These data suggest that miR-135a-5p is a direct target of DANCR, and KLF8 may participate in DANCR-mediated regulation of TSCC malignant phenotypes.

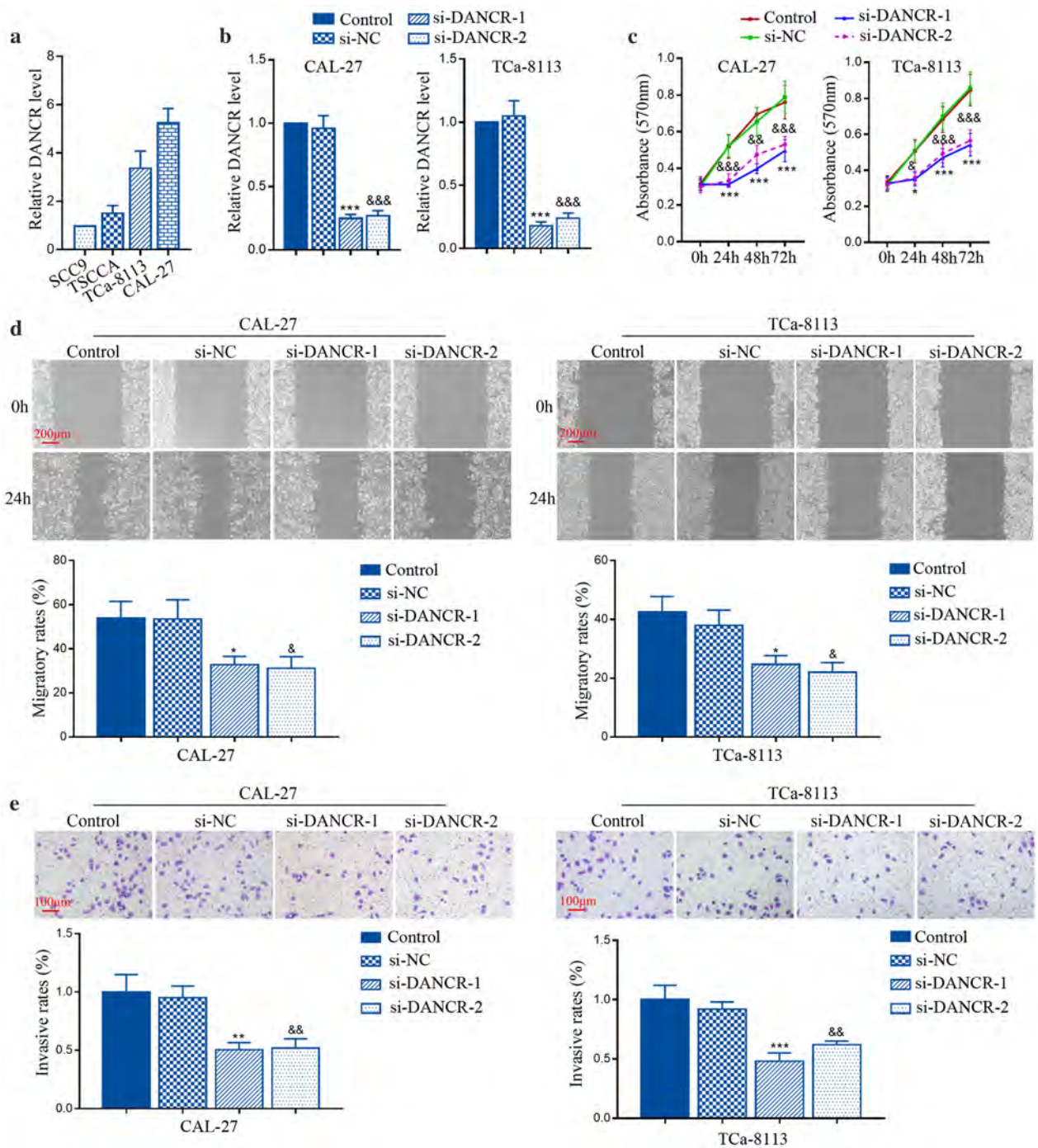


Fig. 1 DANCR knockdown suppressed the proliferation, migration and invasion in vitro. **a** Relative expression of DANCR was detected by qRT-PCR in different TSCC cell lines. **b** CAL-27 and TCa-8113 cells were transfected with siRNAs against DANCR. The relative expression of DANCR was detected by qRT-PCR. **c** The viability of CAL-27 and TCa-8113 cells was assessed by MTT assay. **d**, **e** The migration and invasion of CAL-27 and TCa-8113 cells was determined using wound healing assay and transwell assay, respectively. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$, versus to si-NC

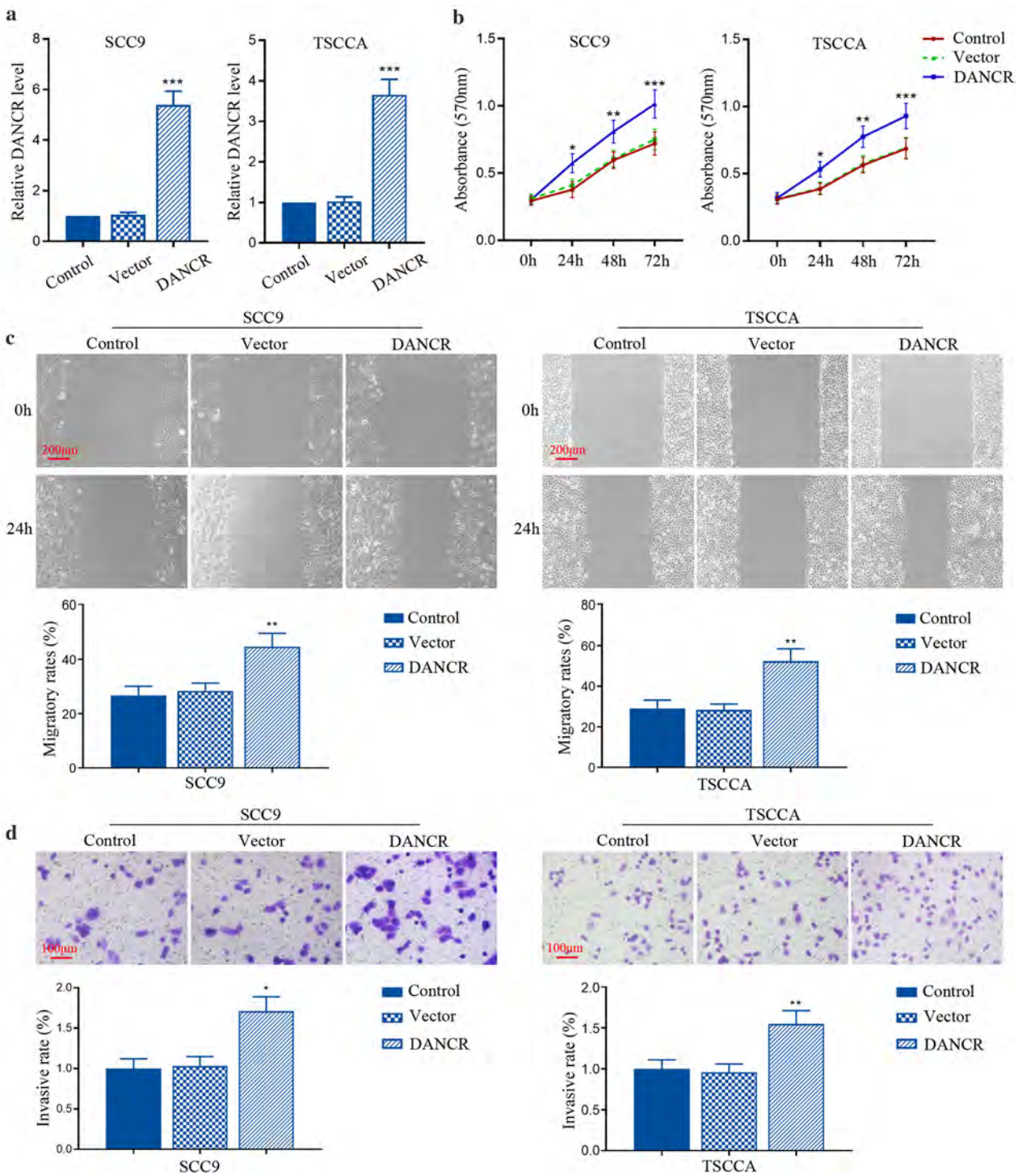


Fig. 2 DANCR overexpression promoted the proliferation, migration and invasion in vitro. **a** SCC9 and TSCCA cells were transfected with pcDNA3.1 vector expressing DANCR. The relative expression of DANCR was detected by qRT-PCR. **b** The viability of SCC9 and TSCCA cells was assessed by MTT assay. **c**, **d** The migration and invasion of SCC9 and TSCCA cells was determined using wound healing assay and transwell assay, respectively. *p < 0.05, **p < 0.01, ***p < 0.001, versus to vector

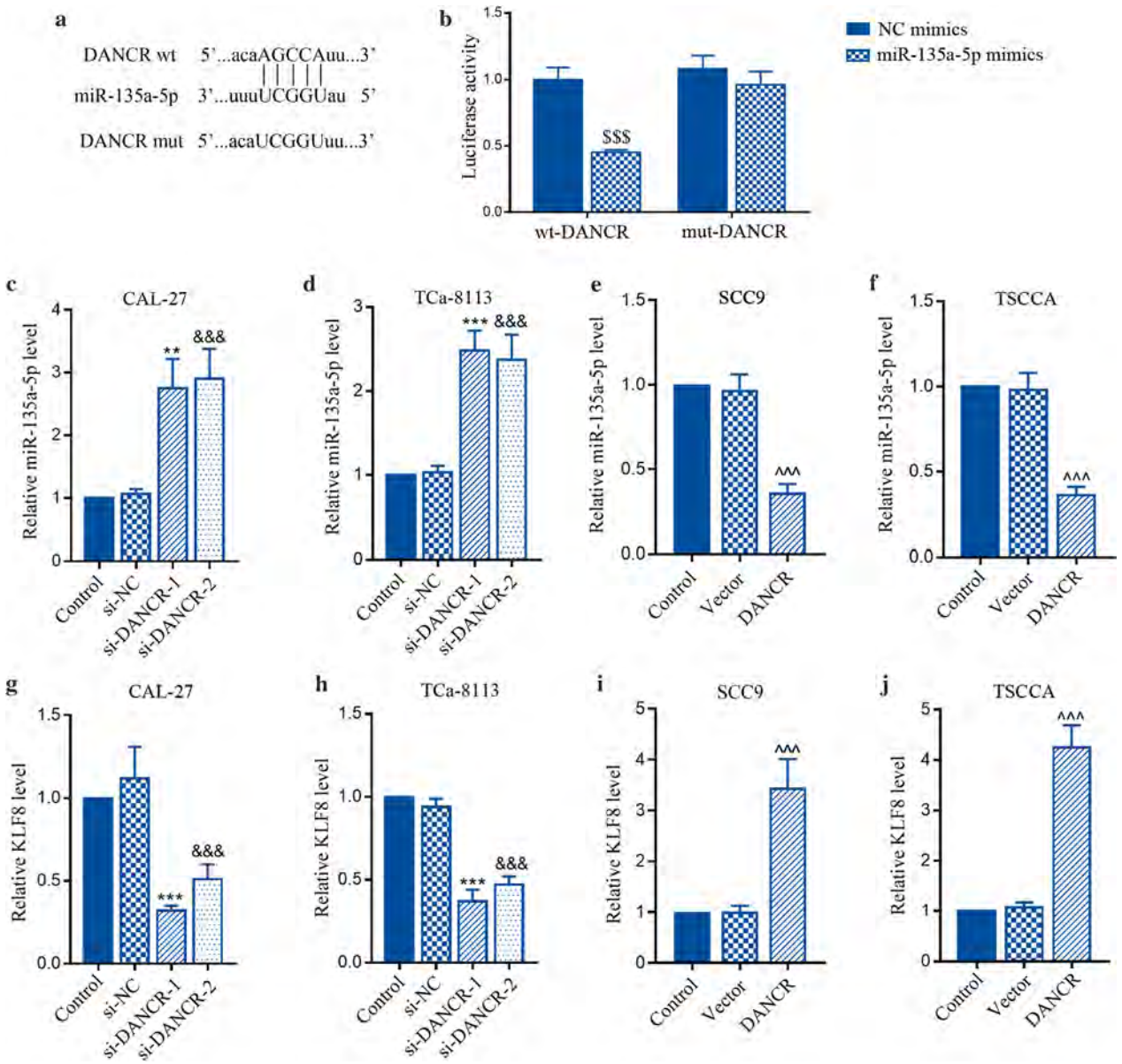


Fig. 3 DANCR targeted miR-135a-5p to regulate KLF8 expression in vitro. **a** Sequence alignments of DANCR with potential targeting sites of miR-135a-5p. **b** Luciferase reporter assay was performed to verify the binding effect between DANCR and miR-135a-5p. **c**–**f** Relative expression of miR-135a-5p was examined by qRT-PCR in CAL-27 cells (**c**), TCa-8113 cells (**d**), SCC9 cells (**e**) and TSCCA cells (**f**). **g**–**j** Relative expression of KLF8 was detected using qRT-PCR in CAL-27 cells (**g**), TCa-8113 cells (**h**), SCC9 cells (**i**) and TSCCA cells (**j**). $^{\$\$\$}p < 0.001$, versus to wt-DANCR + NC mimics. $^{**}p < 0.01$, $^{***}p < 0.001$; $^{\&\&\&}p < 0.001$, versus to si-NC. $^{\wedge\wedge\wedge}p < 0.001$, versus to Vector

MiR-135a-5p overexpression suppressed tumor cell progression and KLF8 expression in TSCC cell lines

Then we found that miR-135a-5p expression in SCC9 and TSCCA cells was higher than that in TCa-8113 and CAL-27 cells (Fig. 4a). To further investigate the role of miR-135a-5p, its specific mimics were further carried out. It obviously confirmed that miR-135a-5p expression was increased by its mimics in CAL-27 and TCa-8113 cells (Fig. 4b). The results in Fig. 4c–e showed that overexpression of miR-135a-5p reduced viable cells, shortened migratory distance and decreased invasive cells in CAL-27 cells and TCa-8113 cells. In addition, KLF8 mRNA and protein expression were also suppressed by miR-135a-5p (Fig. 4f, g). All results indicate that miR-135a-5p may protect against TSCC malignant phenotypes with the involvement of KLF8 suppression.

(See figure on next page.)

Fig. 4 MiR-135a-5p overexpression suppressed tumor cell progression and KLF8 expression in vitro. **a** Relative expression of miR-135a-5p in different TSCC cell lines was examined using qRT-PCR. **b** Relative expression of miR-135a-5p was measured in CAL-27 and TCa-8113 cells transfected with miR-135a-5p mimics by qRT-PCR. **c** The viability of CAL-27 and TCa-8113 cells was measured using MTT assay. **d**, **e** The migration and invasion of CAL-27 and TCa-8113 cells was examined using wound healing assay and transwell assay, respectively. **f** Relative expression of KLF8 mRNA was detected in CAL-27 and TCa-8113 cells using qRT-PCR. **g** Relative expression of KLF8 protein was measured using western blot in CAL-27 and TCa-8113 cells. *p < 0.05, **p < 0.01, ***p < 0.001, versus to NC mimics

DANCR knockdown repressed tumor cell progression and KLF8 expression by targeting miR-135a-5p in TSCC cell lines

Although miR-135a-5p had been identified to target DANCR and be beneficial for TSCC progress, whether miR-135a-5p was responsible for the effects of DANCR on tumor malignancies was unclear. As illustrated in Fig. 5a, the reduction of viable cells by DANCR knockdown was enhanced by miR-135a-5p inhibitor. Furthermore, inhibition of miR-135a-5p reversed si-DANCR-mediated suppression of cell migration and invasion (Fig. 5b, c). It is well-known that matrix metalloproteinase (MMP) family proteins are main biomarkers for tumor invasion and metastasis. Results in Fig. 5d showed that the decrease of MMP-2 and MMP-9 protein levels induced by DANCR silence was partially increased by miR-135a-5p inhibitor. In addition, we found that the reduction of KLF8 in si-DANCR cells was increased by miR-135a-5p inhibitor (Fig. 5e). Together the results further suggest that DANCR/miR-135a-5p may modulate TSCC progression by the regulation of KLF8.

MiR-135a-5p inhibition exacerbated tumor cell progression through activating KLF8 in TSCC cell lines

Next, we further elucidated whether KLF8 was responsible for the regulatory function of DANCR/miR-135a-5p in SCC9 cells using its specific siRNA. Expectedly, miR-135a-5p inhibitor-induced increase of KLF8 was suppressed by the siRNA of KLF8 itself (Fig. 6a). Knockdown of KLF8 attenuated the effects of miR-135a-5p inhibitor on the proliferation, migration and invasion of SCC9 cells (Fig. 6b–d). Similarly, the indicators for tumor development and progression, MMP-2 and MMP-9 were also inhibited by KLF8 silencing (Fig. 6e), which just proved the alterations of tumor malignancies at molecular level. Collectively, these findings demonstrate that KLF8 is responsible for the regulation of DANCR/miR-135a-5p on TSCC progression.

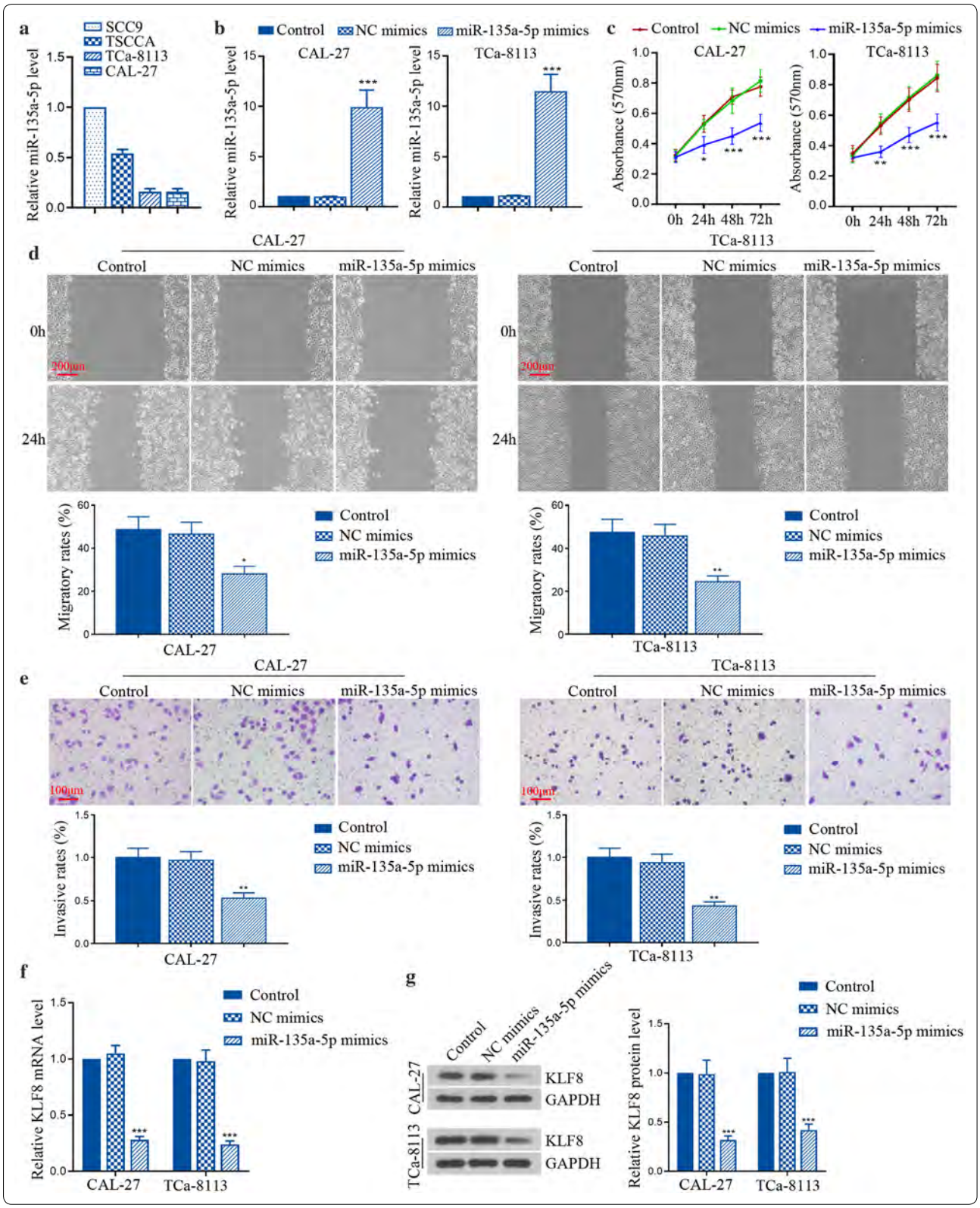
DANCR knockdown blocked the tumor formation in vivo involving KLF8 activation

To test the role of DANCR in tumor growth in vivo, CAL-27 or TCa-8113 cells were stably transfected with shRNAs and injected subcutaneously into the right flank of axilla of nude mice. As shown in Fig. 7a, b, it showed that the tumor size and weight could be suppressed by knockdown of DANCR. At molecular level, the expression of MMP-2 and MMP-9 in tumor tissues was also reduced by DANCR inhibition (Fig. 7c). In addition, as shown in Fig. 7d, e, both western blot and immunofluorescence staining demonstrated that a remarkable down-regulation of KLF8 was induced in tumor tissues stably transfected with DANCR shRNA. Overall, these in vivo results show that DANCR may activate the expression of KLF8 and MMPs to affect TSCC tumor growth.

Discussion

Increasing lncRNAs have been revealed to be implicated in the development and progression of various cancers, including TSCC [7, 8, 22]. In this work, DANCR was showed to act as an oncogenic gene to facilitate the proliferation, migration and invasion of TSCC cells through the loss or gain of DANCR. Furthermore, miR-135a-5p was demonstrated to be complementary with DANCR and negatively regulated by DANCR. Overexpression of miR-135a-5p prevented the malignant phenotypes of TSCC cells and reduced the expression of KLF8. Inhibition of miR-135a-5p mediated the protective effects of DANCR silence on TSCC cells. KLF8 was responsible for the regulatory role of miR-135a-5p through modulating MMP-2/9 expression.

Previous reports showed that lncRNA DANCR was high-expressed in esophageal cancer [23], liver cancer [10], colorectal cancer [24], prostate cancer [11], retinoblastoma [25] and so on, which indicated its potential correlation with the poor prognosis of patients. Evidence demonstrated that DANCR enhanced the migration and invasion of prostate cancer cells or gastric cancer cells through impeding TIMP2/3 expression [11] or lncRNA-LET [26]. Jiang et al. suggested that the initiation and progression of osteosarcoma was affected by DANCR via competitively binding to miR-33a-5p [27]. In NSCLC cells, DANCR was found to target miR-758-3p to regulate cell proliferation, migration and invasion [28]. However, up to now, the functional significance of DANCR in the progression of TSCC still requires to be clarified. In this study, the gain- and loss-of-function experiments showed that DANCR could enhance the proliferation, migration and invasion of TSCC cells. The in vivo results further demonstrated that inhibition of DANCR prevented


a
SCC9
TSCCA
TCa-8113
CAL-27
Relative miR-135a-5p level
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Relative miR-135a-5p level
c
Control
NC mimics
miR-135a-5p mimics
CAL-27
TCa-8113
Absorbance (570nm)
0h 24h 48h 72h
d
CAL-27
TCa-8113
Control
NC mimics
miR-135a-5p mimics
0h
24h
200μm
Migratory rates (%)
e
Invasive rates (%)
100μm
f
Relative KLF8 mRNA level
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KLF8
GAPDH
Relative KLF8 protein level

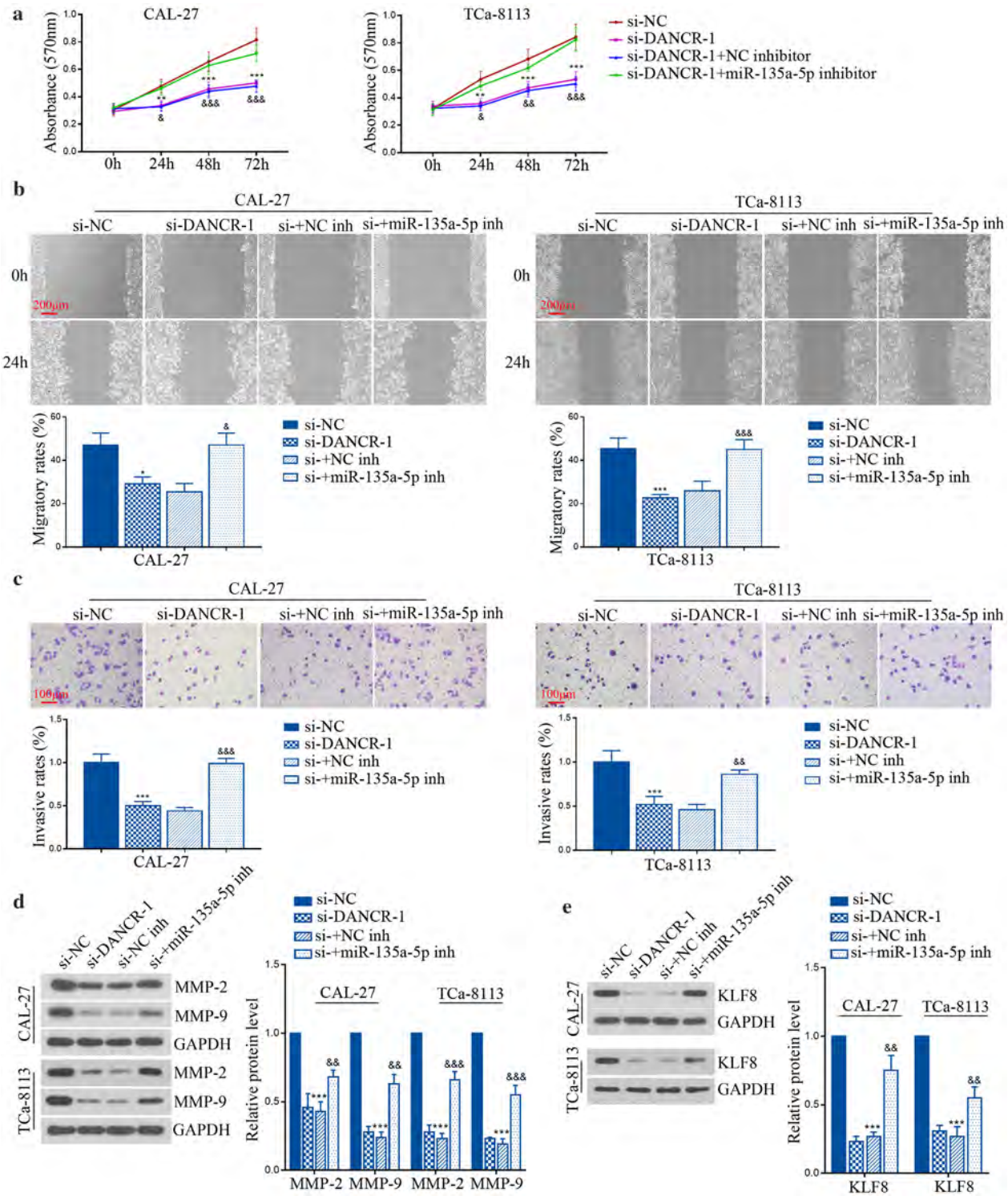

Fig. 5 DANCR knockdown repressed tumor cell progression and KLF8 expression by targeting miR-135a-5p in vitro. **a** The viability of CAL-27 and TCa-8113 cells was measured using MTT assay. **b**, **c** The migration and invasion of CAL-27 and TCa-8113 cells were detected by wound healing assay and transwell assay, respectively. **d** Relative expression of MMP-2 and MMP-9 protein was determined by western blot in CAL-27 and TCa-8113 cells. **e** Relative expression of KLF8 protein was detected using western blot in CAL-27 and TCa-8113 cells. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, versus to si-NC; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$, versus to si-DANCR-1 + NC inhibitor

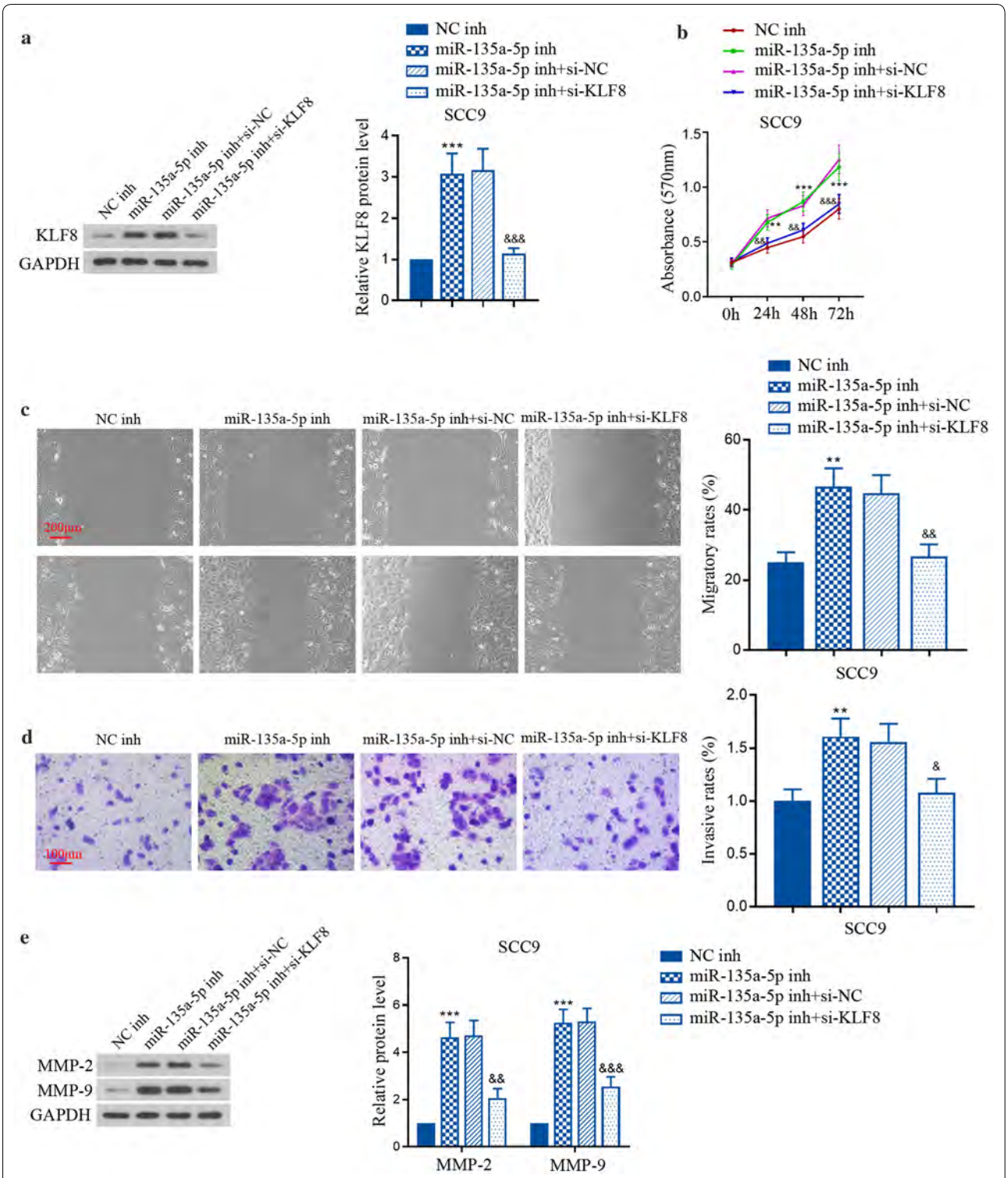

Fig. 6 MiR-135a-5p inhibition exacerbated tumor cell progression through activating KLF8 in vitro. **a** Relative expression of KLF8 protein in SCC9 cells was tested by western blot. **b** The viability of SCC9 cells was assessed by MTT assay. **c**, **d** The migration and invasion of SCC9 cells was measured using wound healing assay and transwell assay, respectively. **e** Relative expression of MMP-2 and MMP-9 protein in SCC9 cells was examined using western blot. $^{**}p < 0.01$, $^{***}p < 0.001$, versus to NC inhibitor; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$, versus to miR-135a-5p inhibitor + si-NC

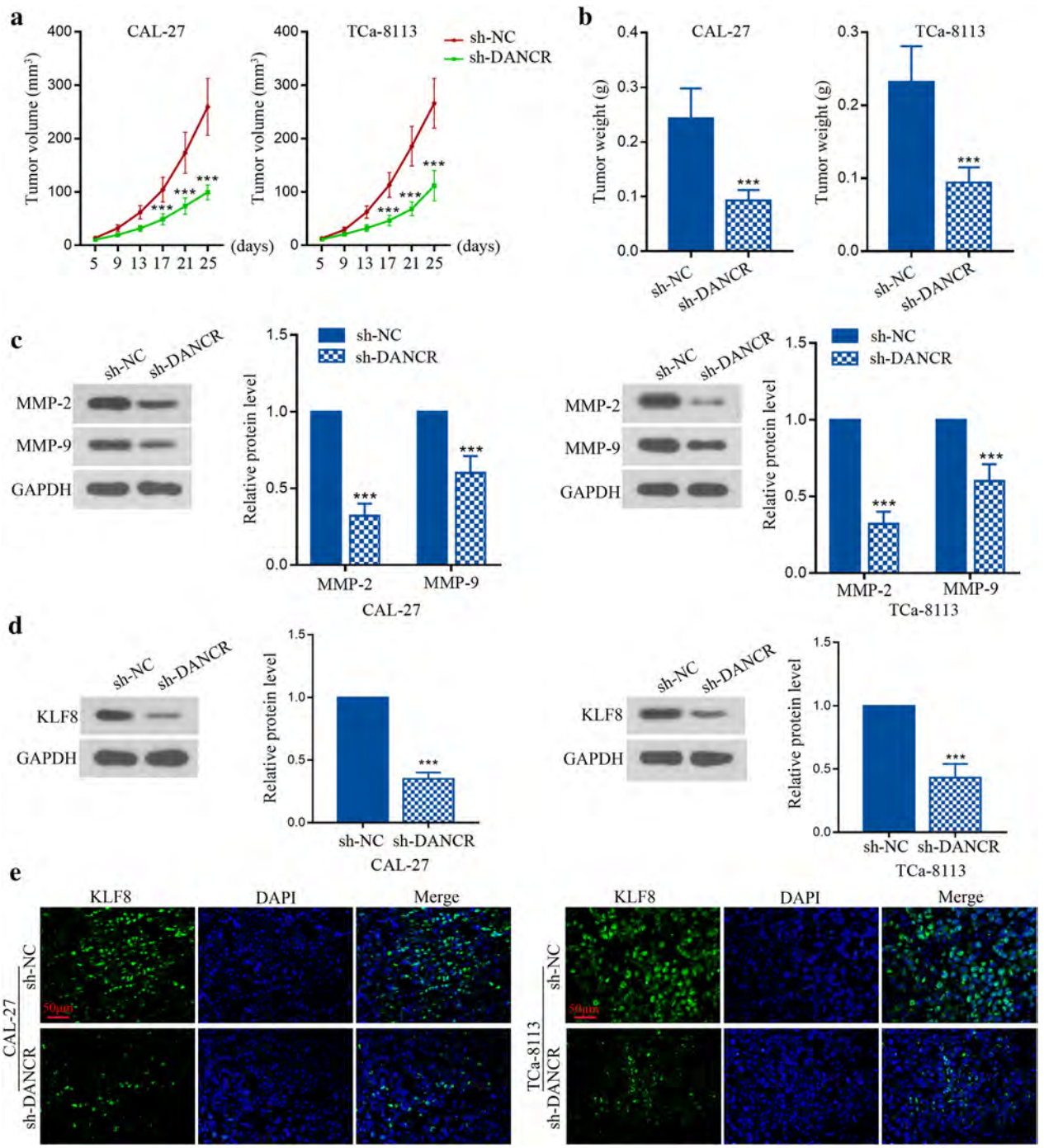

Fig. 7 DANCR knockdown blocked the tumor formation in vivo involving KLF8 activation. CAL-27 or TCa-8113 cells transfected with shRNA against DANCR were inoculated subcutaneously into the nude mice. Xenografts were measured every 4 days with a caliper. **a** Tumor volumes were measured every 4 days. **b** Mice were sacrificed after 25 days, and xenograft tumors were excised and weighed. **c** Relative expression of MMP-2 and MMP-9 protein in tumor tissues was measured by western blot after 25 days. **d** Relative expression of KLF8 protein in tumor tissues was examined by western blot after 25 days. **e** Immunofluorescence staining was performed to investigate KLF8 immunoreactive materials in tumor tissues after 25 days. ***$p < 0.001$, versus to sh-NC

the tumor growth, which indicates the oncogenic role of DANCR in TSCC tumorigenesis.

To the best of our knowledge, this was the first report about the role of DANCR in the progression of TSCC. Emerging references suggested that lncRNAs might function as "sponge" of miRNAs to participate in multiple biological processes. For instance, lncRNA ZFAS1 activated the expression of ZEB1, MMP-14 and MMP-16 to promote tumor growth and metastasis by sponging miR-150 in hepatocellular carcinoma [29]. Wang et al. reported that DANCR facilitated ROCK1-mediated malignant biological behaviors through decoying both miR-335-5p and miR-1972 in osteosarcoma [30]. In this current study, functional experiments indicated that miR-135a-5p overexpression protected against the proliferation, migration and invasion of TSCC cells in vitro, which was showed to directly target DANCR. The inhibitory effects of DANCR silence on TSCC progress could be rescued by silencing miR-135a-5p. Altogether, this study shows that miR-135a-5p serves as a "sponge" miRNA of DANCR to prevent the progression of TSCC.

MiRNAs modulate gene transcription and expression by directly targeting the 3′ UTR of mRNAs, and lncRNAs may exhibit sponging effects on miRNAs during tumor progression. DANCR had been described to competitively bind miR-149 to positively regulate MSI2 expression and promote tumor malignant phenotypes in the pathogenesis of bladder cancer [31]. Although KLF8 expression was altered by DANCR and miR-135a-5p, whether KLF8 was the downstream effector of DANCR/miR-135a-5p to mediate the regulation of TSCC progression was not well understood. Knockdown of KLF8 attenuated the effect of miR-135a-5p inhibitor on TSCC cell proliferation, migration and invasion. More importantly, KLF8 was reported to be a direct target of miR-135a-5p to inhibit NSCLC cell migration, invasion and EMT process by Shi et al. [16]. Together, these results suggest that DANCR/miR-135a-5p axis affects the malignancies of TSCC by the regulation of KLF8.

In addition, MMP is a classical zinc-dependent endopeptidase to affect cell proliferation, angiogenesis, and tumor invasion and metastasis through the degradation of extracellular matrix [32, 33]. MMP-2 and MMP-9 had been demonstrated to be important prognostic biomarkers in diverse cancers, such as breast cancer, colorectal cancer, and NSCLC [34–36]. Considering that KLF8 was highlighted to bind the promoter of MMP-9 to induce its expression and stimulate cancer invasion [37, 38], thus we further examined the alterations of MMPs in the downstream of KLF8. Our data showed that the expression of MMP-9 and MMP-2 was altered by DANCR/miR-135a-5p/KLF8 axis, which just further proved the regulatory network on tumor malignancies from the point of molecular level. Therefore, we conclude that DANCR serves as a "sponge" of miR-135a-5p to activate KLF8/MMP-2/9 signaling pathway, thus stimulating the development and progression of TSCC.

Conclusion

In conclusion, this present study develops a novel insight that the TSCC tumor progression may be regulated by DANCR/miR-135a-5p/KLF8 axis. To the best of our knowledge, DANCR is suggested to function as a diagnostic biomarker of TSCC for the first time, which may provide new therapeutic targets for the prevention and treatment of TSCC.

Abbreviations

TSCC: tongue squamous cell carcinoma; HNSCC: head and neck squamous cell carcinoma; KLF8: Kruppel-like Factor 8; DANCR: differentiation antagonizing non-protein coding RNA; ceRNAs: competing endogenous RNAs; MMP: matrix metalloproteinase.

Acknowledgements

Not applicable.

Authors' contributions

YL and YZ conceived and designed this study. YZ, BZ and XM performed the experiments. YY and JH analyzed the data; YZ and YL contributed reagents and materials. YZ wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

The procedures of animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals. The ethical approval was obtained from School of Stomatology, China Medical University Committee (No. G2018007).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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専攻種別	□論文博士		☑課程博士		

研究者評価（指導教官記入欄）

成績状況	優　良　可　不可 学業成績係数=	取得単位数 取得単位数 10／取得すべき単位総数 20
学生本人が行った研究の概要	劉雨桐さんは、外国人医療通訳者と日本人医療通訳者それぞれの特性を明らかにし、さらにその特性が医療通訳の質にどのような影響を及ぼすかを研究テーマとしている。 研究は次の三つのステップに分けて調査・分析を進めている。 ① 医療通訳の利用側（医療従事者）の視点からアプローチし、医療通訳に対する考え方を捉え、日中医療システムや医療文化の違いを明確にする。 ② 医療通訳者の視点からアプローチする。外国人医療通訳者と日本人医療通訳者の特性を聴取し、通訳の質を評価してもらう。 ③ 上記①②の調査で得られたデータを比較分析を行い、客観的な視点と主観的な視点を合わせて各特性が通訳の質にどのような影響を与えるかを明らかにする。 現在はステップ①の調査研究を順調に実施しているところであり、ステップ②③の研究も着実に進めており、医療通訳分野に寄与できる成果が期待される。	
総合評価	【良かった点】 研究テーマはこれまでありそうでなかったものを取り上げていて、その研究成果は日中双方の医療現場に寄与できるものだと期待している。 研究は三つのステップに分けて進められており、着実に実行可能な研究計画だと評価したい。現在初年度を終えたところで、計画通り日中両国の医療現場を熟知する医療従事者にインタビューし、生のデータを得られたことは、この研究分野において初めてのことであり、医療通訳研究に貴重なデータを提供できると考える。さらにインタビューの分析を通して、利用者側の医療従事者の視点から見る日中の医療システム、診療文化の違いや通訳の役割、問題点などを明らかにできたことは大いに評価できる。 その他、指導教官が所属する厚労省科研費研究班の感染症医療通訳研修において、劉雨桐さんはロールプレイシナリオの翻訳や患者役を務め、中国の技能実習生へのアンケートの翻訳なども積極的にこなし、こうした実務経験を積むことで研究に深みを持たせたと評価する。	
	【改善すべき点】 今年度は学会での発表や論文の発表はできていないので、次年度は日中双方の学会で発表の機会があればと考える。	
	【今後の展望】 今年度は医療通訳の利用側である医療従事者へのインタビューはできたが、来年度は日本人医療通訳者と中国人の医療通訳者へのインタビューを行い、自ら立てた仮説を検証する。また、その成果を学会で発表し、論文を投稿してほしいと思う。	
学位取得見込	研究計画を年度ごとに着実に行っていけば、順調に学位の取得は可能だと考える。	

評価者（指導教官名）　宮首弘子　印

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研究者用

第41期　　研究者番号：　G4106　　作成日：2020年2月27日

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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）

近年グローバル化の流れによって、来日観光客は増加の一途をたどっている。また、国連人口部は、少子・高齢化による人口減少と将来的な労働力不足が問題になっている日本に対して、1995年の生産年齢人口を維持するためには2000年から2050年の間に毎年新たに67万7千人の移民を受け入れる必要があると指摘している。実際近年では外国人労働者、日系人、国際結婚配偶者が急増し、その一部が日本社会に定着する傾向がある。こうした背景の下で、訪日・在日外国人患者も受診しやすい医療環境の整備もますます重要となり、その一環として、質の高い医療通訳の確保が急務とされている。

現在、医療通訳者は医療従事者と同じく患者の生命と健康を守る医療チームの一員とみなされ、様々な角度から研究されているところであるが、その中で、患者側と同じ文化を持つ外国人医療通訳者と医者側と同じ文化を持つ日本人医療通訳者の違いに注目し、外国人医療通訳者が直面する困難とその対処を研究する方向がある。田中郁子・柳澤理子は論文「外国人医療通訳者の体験した困難とその対処」で、医療隠語、略語など日本人ならば非医療従事者でも知っている言葉が分からない外国人医療通訳者は日本人医療通訳者と比べて、医療システムへの理解に欠けていると指摘している。しかし、医療システムや医療文化など背景知識の差によって医療通訳者の特性にどのような影響をもたらすかはまだ研究されていない。

本研究の目的は、外国人医療通訳者と日本人医療通訳者それぞれの特性を明らかにし、こうした特性が医療通訳の質にどのような影響を及ぼすかを研究することである。

2）戦略（Approach）

より客観的な研究結果を得るために、本研究は三つのステップの調査・分析を踏まえて結論に導く。

現在の見通しを以下のように記述する。

① 医療通訳の利用側（医療従事者）の視点からアプローチし、医療通訳に対する考え方を捉え、日中医療システムや医療文化の違いを明確にする。

② 医療通訳者の視点からアプローチする。外国人医療通訳者と日本人医療通訳者の特性を聴取し、通訳の質を評価してもらう。

③ 上記①②の調査で得られたデータを基に比較分析を行い、客観的な視点と主観的な視点を合わせて各特性が通訳の質にどのような影響を与えるかを明らかにする。

上記三つのステップを３年計画とし、１年目は計画通り、医療従事者を対象とするインタビュー調査を進めた。

１年目の研究では医療通訳の利用側である医療従事者の視点から外国人医療通訳者と日本人医療通訳者の役割意識や特性を比較し、それぞれの優位性と問題点を抽出する。「外国人医療通訳者と日本人医療通訳者の特性の相違点」、「医療通訳に対する考え方」、「医療通訳介入の効果」、「医療通訳者として大事な要素」などの問題を明確化することによって、医療通訳利用側がどのような医療通訳者を求めるかを把握する。

また、医療システムと医療文化の差は医療通訳者にとって重要な背景知識であることに注目する。医療通訳の現場で医療通訳者として医療システムと医療文化において情報量の差を埋めないと、双方の誤解を招く危険性がある。医療場面の誤訳や異文化ギャップを避け、医療通訳の質を向上させるため、このような背景知識を明確にすることは大変有意義であると考えられる。

さらに本研究では医療通訳者としてどのように医療システムの違いがもたらすハンデを乗り越えるかを考察する。現在日中医療システムの相違点を比較する先行研究はまだ不十分である。その研究方向の空白を埋めるために、本研究は日本の医療機関で研究活動をする中国の医療従事者が気づいた「日中医療システムの相違点」、「日中医療理念の相違点」、「日中医療文化の相違点」、「日中医療保険の相違点」、「外国人が受診する際に面する困難」などの問題をまとめて分析する。

3）材料と方法（Materials and methods）

本研究は質的研究方法（グラウンデッド・セオリー・アプローチ）に基づいて研究を進め、仮説生成を目指している。そのため、日中双方の医療現場を知る医療従事者からデータを取ることは何より説得力があると考える。したがって、中国の医療機関での勤務経験があり、博士号取得のためあるいは共同研究を行うために日本の医療機関で研修をした医療従事者を対象に設定し、半構造化面接を行う。対象者の選定にあたっては本人からの同意を得る。

１．研究概要（2）

インタビューのリサーチクエスチョンは「日中医療システムの相違点」、「日中医療文化の相違点」、「外国人医療通訳者と日本人医療通訳者の特性の相違点」、「医療通訳に対する考え方」、「医療通訳介入の効果」、「医療通訳者として大事な要素」などの問題である。インタビューを行う際に個室で聴取する。

具体的にはインタビューの内容を録音し、逐語録を作成した上、研究協力者に内容をチェックしてもらってから日本語に訳す。データを数件収集後、データ全体に対するコーディング化作業（長いインタビューデータを細かく分け、抽象度を上げて単語や箇条書きのラベルにする作業）を始める。次に多様に出現したラベルをまとめ、そこから共通性のある結論を見つけ出す。その結論に適切なデータを収集するため、数件のデータ収集後に分析作業を行い、再びデータ収集に戻ってデータの備蓄を行う。螺旋状にデータの収集と分析を行うことによって、結論を精緻化する。

4）実験結果（Results）

1年目は計画通り、9人の医療従事者（医師８人、研究者1人）を対象にインタビューを行い、計400分の音声データを収集し、８万字のテキストデータを書き起こした。

今までのインタビュー調査から、いくつかの傾向がうかがえる。

① 医療通訳の利用側（医療従事者）は医療通訳者の重要性を認めていて、機械通訳の普及はまだ難しい。

② 患者と同じ文化背景を持つ外国人医療通訳者と医療従事者と同じアイデンディティを持つ日本人医療通訳者はそれぞれ優位性と問題点がある。患者のメンタルケアなどを配慮する上で、患者と同じ国籍の外国人医療通訳者が望ましいという声が多くある一方、医療従事者から情報をたくさん聞き出す日本人医療通訳者の優位性も無視できない。また、中国独特の柔軟性に富む医療事情から考えると、患者の文化背景やニーズをよく理解する医療通訳者の存在は欠かせない。

③ 医療従事者が求める良い通訳者の役割と評価要素は、今多くの研究者に使われている評価基準と差異があることが浮き彫りになった。例えば通訳教育の場によく問われる「発音」、「流暢性」、「ロジック」などの要素は医療通訳の利用側に見ればそれほど大きな問題ではない。この情報を踏まえて、「医療知識」、「患者への思いやり」、「やさしい態度」、「臨機応変能力」、「円滑なコミュニケーション能力」などの要素を加えて、中国事情と医療場面の性質に相応しい医療通訳の評価基準の構築を試みている。

④ 日中医療システムの相違点は、「受診・入院の仕組み」、「病院の管理システム」、「医療理念」、「医療文化」、「医療保険」5つのカテゴリーに分けられると考えられる。外国人患者が受診の際に直面する困難以外に、患者が受けやすい文化ショックや医療トラブルになりがちな考え方を分析する上、医療通訳の質を高める方法を考察する。さらに、日中医療の相違点は外国人医療通訳者と日本人医療通訳者の差異が生じる背景である可能性が高いと考えている。その関連性は次の研究で検証していく。

5）考察（Discussion）

現在、本研究ではステップ①として医療通訳を利用する医療従事者の視点から医療通訳に存在する諸問題を考察し、医療通訳者にとって重要な背景知識、日中医療システムの相違点を明らかにした。その結果、医療通訳に関する先行研究の一部の結論が検証された。医療通訳者は現段階で機械に代替できない存在であるため、医療通訳の質を高める研究に力を入れる価値がある。そして、医療通訳に対する新しい観点も浮き彫りになった。今年の研究結果に基づいて作成した評価基準を用いて、抽象的なデータ医療通訳の質を量的な数値に表すことが期待できる。

今年の研究は今後重要な土台となる。これからは計画通りに外国人医療通訳者と日本人医療通訳者に対するインタービューを行い、それぞれの特性と通訳の質の関連性を研究するステップ②に踏み込んでいきたいと考える。

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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	なし				
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 interna

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

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（署名） 宮首弘子

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 41 期　研究者番号：G4107　作成日：2020 年 3 月 日

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研究先（指導教官）	金沢医科大学糖尿病・内分泌内科（古家 大祐教授）				
研究テーマ	SGLT-2 阻害薬と糖尿病性腎臓病 The singnificance of sirtuin and SGLT-2 against diabetic nephropathy				
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	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	培養不死化ヒト近位尿細管細胞株 HK-2 細胞を正常血糖(5mM)あるいは高血糖(25mM)に 48 時間に曝すと、HIF-1α の高発現を介して、炎症性サイトカイン(IL-1β、TNFα、IL-6)の産生が増強した。しかし、これら炎症性サイトカインの増強は、同細胞を siRNA ATG-5 あるいは SGLT2 阻害薬 dapagliflozin(50μM)にて処理すると軽減された。さらに、高血糖に曝すと AMPK 活性の低下、PGC1α の低下、ミトコンドリア量の低下がみられたが、dapagliflozin(50μM)にて処理すると回復した。また、高血糖に曝すとSGLT2発現の増強が生じたが、その強発現は dapagliflozin 濃度依存性に抑制された。これらの結果は、高血糖によるミトコンドリア障害と炎症が、SGLT2 によって惹起されるが、SGLT2 阻害薬 dapagliflozin によってミトコンドリア障害と炎症が改善することを示唆する。	
総合評価	【良かった点】 2019 年 4 月から実験にも慣れており、上述した成果を挙げてきた。来日以来、当科の留学研究者、大学院生と密接に学術的交流を図り、実験手技も確実に向上している。 研究者としてだけでなく、ヒトとして非常に勤勉でかつ努力家であり性格もよい。また。医学研究知識も豊富であり、BBA -Molecular Basis of Disease(IF 4.328)にミトコンドリア機能の維持と糖尿病性腎臓病に関する総説を再投稿している。	
	【改善すべき点】 2019 年 4 月から、上述した培養細胞における実験から成果を挙げてきており、今後、糖尿病モデルラットによる検証が必要である。	
	【今後の展望】 培養細胞の実験結果から SGLT2 の存在によって、ミトコンドリア障害を介して炎症が生じること、さらに、それら変異が SGLT2 阻害薬によって改善することを見出している。今後、糖尿病モデルラットにおいて、培養細胞の自験から得た成果を検証できることを期待している。	
学位取得見込	現在、並行して糖尿病モデルラットの糖尿病性腎臓病に対する、発症予防と進展抑制の SGLT2 阻害薬 dapagliflozin 投与、非投与の実験が進捗している。培養細胞で得られた結果の再検証と、モデル動物の結果が合致する可能性が高く、英語論文作成とが奇異取得の可能性は高いと確信している。	

評価者（指導教官名）　古家　大祐　古家 印

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41期　　研究者番号：G4107　　作成日：2020年3月 1 日

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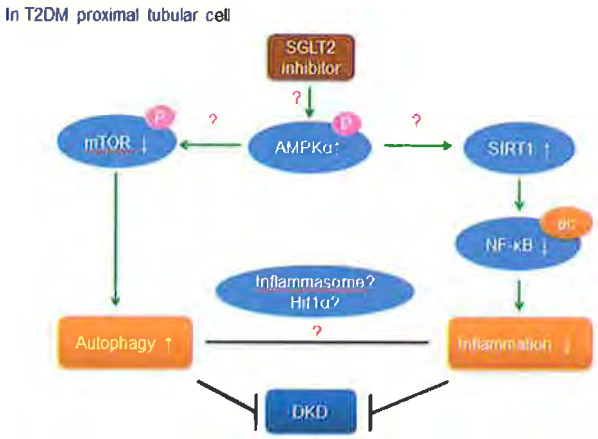
研究テーマ	SGLT-2 阻害薬と糖尿病性腎臓病 The singnificance of sirtuin and SGLT-2 against diabetic nephropathy				
専攻種別	論文博士	☑	課程博士	□	

1．研究概要（1）

1）目的（Goal）
To evaluate protective effects of dapagliflozin against diabetic kidney disease (DKD) focusing on mitochondorial dysfunction related to inflammation and autophagy.

2）戦略（Approach）

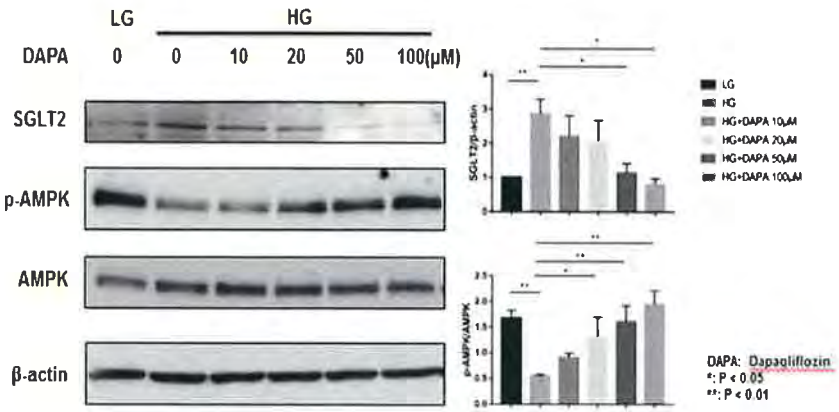


3）材料と方法（Materials and methods）
3.1 Cell culture
Human kidney proximal tubular cells (HK-2 cells) were cultured in 5mM or 25mM glucose modified Eagle' s medium (DMEM) in the presence or absence of dapagliflozin(50μM) for 48h.
3.2 Mitotracker green staining
HK-2 cells were cultured in eight-well culture slides with DMEM in the presence or absence of dapagliflozin for 48h. Then removed the medium and added pre-warmed MitoTracker Green (100nmol/L) for 30 min at 37° C. The mitochondrial staining was observed under a fluorescence microscope.
3.3 Autophagic flux
HK-2 cells were cultured in 5mM, 25mM DMEM in the presence or absence of dapagliflozin or insulin (100nM) for 24h, then incubated with Chloroquine (100μM) for 1h.
3.3 Western blot analysis
Proteins were harvested using radioimmunoprecipitation assay lysis buffer then were boiled at 100° C for 5 min. Lysates were separated on sodium dodecylsulfate-polya-crylamide gels and transferred onto polyvinylidene fluoride membranes by using the semidry method. After blocking with Tris-buffered saline with Tween 20 containing 5% non-fat dry milk, the membranes were incubated with primary antibodies (SGLT2 1:200, p-AMPK 1:1000, AMPK 1:1000, SIRT1 1:500, acetylated-NFkB p65 1:200, NF-kB p65 1:1000, IL-1β 1:500, IL-6 1:500, TNFα 1:1000, p-mTOR 1:1000, mTOR 1:1000, p-S6 1:1000, S6 1:1000, LC3 1:1000) at 4° C overnight. The membranes were washed with Tris-buffered saline with Tween 20 three times and then incubated with HRP-conjugated secondary antibodies for 1h at room temperature. After washing with Tris-buffered saline with Tween 20 three times, the blots were developed with an enhanced chemiluminescence detection system and visualized using an Image-Quant LAS 400 camera system.

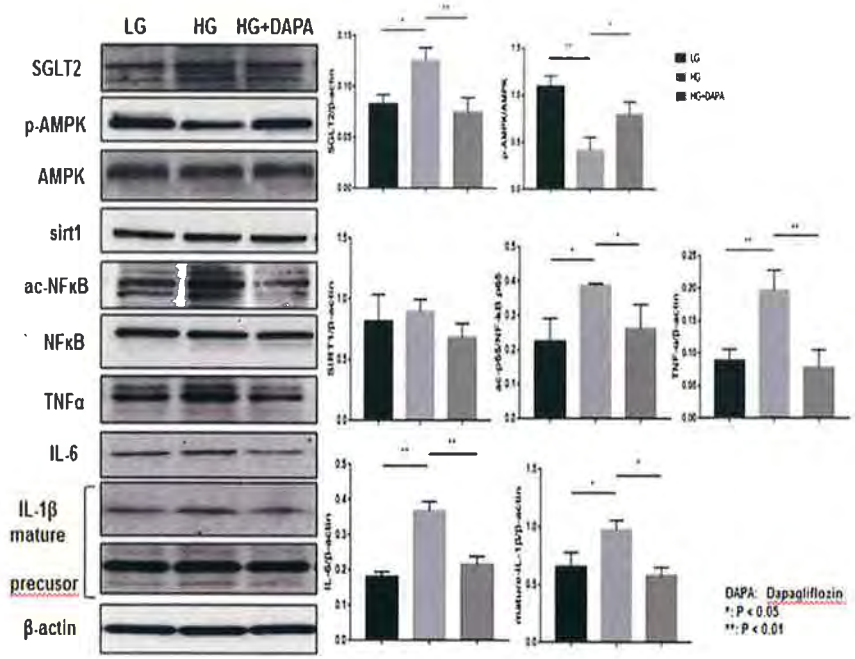
1．研究概要（2）

4）実験結果（Results）

4.1 Dapagliflozin suppressed the expression of SGLT2 and activated p-AMPK in a dose-dependent manner in HG treated HK-2 cells.

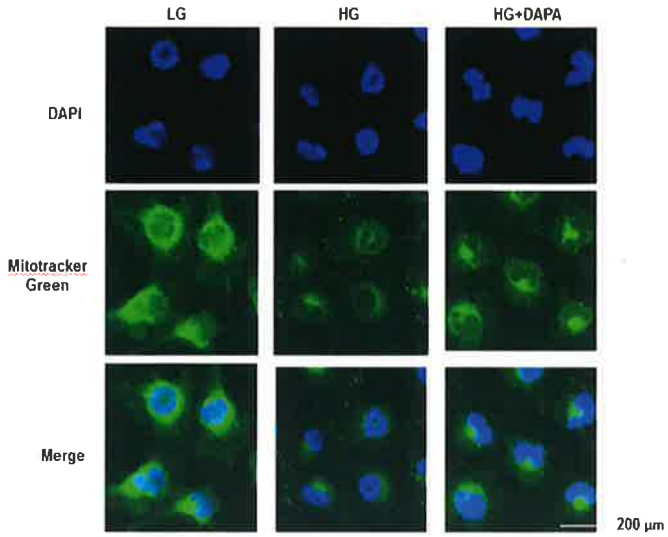


4.2 Dapagliflozin activated AMPK, suppressed the expression of inflammatory cytokines, such as IL-1β, IL-6 and TNFα via activating p-AMPK and deactylating NF-κB via SIRT1.

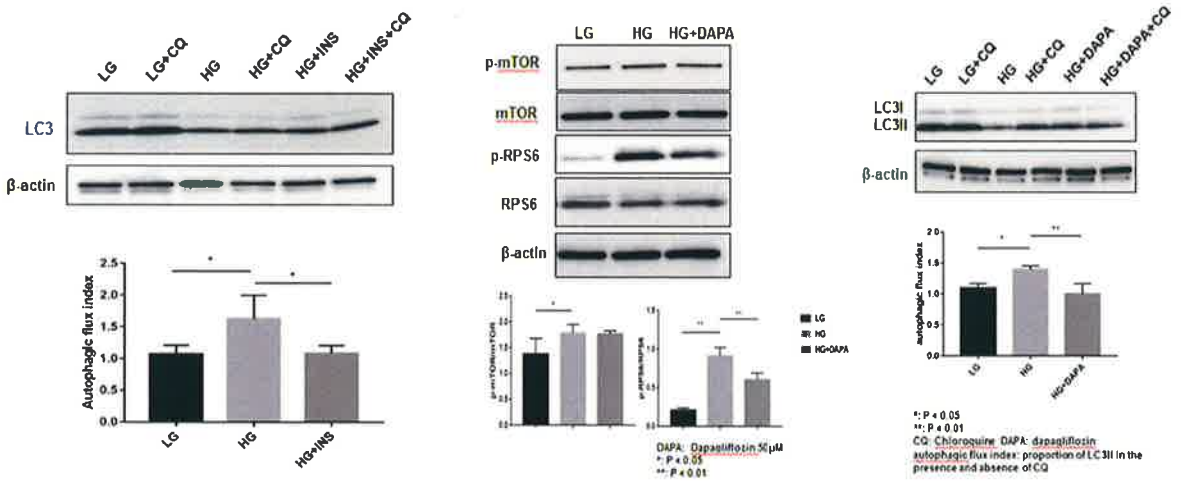


1．研究概要（3）

4.3 Dapagliflozin restored the mass of mitochondria, which were suppressed by HG in HK-2 cells.



4.4 HG induced autophagic flux and insulin suppressed autophagic. Dapagliflozin treatment inhibited mTOR pathway, via activating p-AMPK, but could not induce autophagic flux in HG treated HK-2 cells.



5） 考察（Discussion）

The pathogenesis of T2DM and DKD involve in multiple mechanisms. Among them mitochondrial dysfunction plays a central role and is closely related to inflammation, oxidative stress, and impaired autophagy [1, 2]. Previous reports have shown that members of the mammalian Sirtuin family play a crucial role in the regulation of mitochondrial quality control [1, 3, 4]. As the most widely studied member of the Sirtuin family, SIRT1 is a NAD+-dependent deacetylase, it deacetylates multiple transcription factors and proteins, which involves mitochondrial biogenesis [5], oxidative stress[6], inflammation [7] and autophagy [8].

Sodium-glucose cotransporter 2(SGLT2) inhibitors are effective antidiabetic drugs that have been confirmed to reduce high glucose independent of insulin and protect against progression of DKD [9]. In db/db mice, SGLT2 expression increased with concomitant decreases in SIRT1, SGLT2 inhibitor canagliflozin activated AMP-activated protein kinase (AMPK) and SIRT1 [10].

1．研究概要（4）

In our study, we found that HG induced inflammatory cytokines, such as IL-1β, IL-6 and TNFα. Dapagliflozin inhibited inflammation via activating p-AMPK and deactylating NF-κB through SIRT1 in HK2 cells. However, the protein level of SIRT1 was no difference in these groups. These results were consistent with our previous research in db/db mice, a T2DM animal model [6]. We speculated that dapagliflozin may have an effect on the activity of SIRT1 without changing its protein expression. The intracellular NAD+/NADH ratio and activity of SIRT1 are needed to detect in our following experiment.

Our data also showed autophagic flux was increased in HG condition and suppressed in the presence of insulin. Dapagliflozin restored p-AMPK, then suppressed mTOR pathway. These were consistent with previous study [11] Proximal tubule autophagy differs in Type 1(insulin deficiency) and 2 Diabetes (insulin resistance). Autophagy is induced in STZ-induced T1DM mice via various cellular stresses such as reactive oxygen species, endoplasmic reticulum and hypoxia, while is suppressed in db/db mice [11]. Our results showed Dapagliflozin could not increase autophagic flux, which is inconsistent with some previous studies [12, 13]. We need to confirm our data in the further experiment and looking for other mechanisms.

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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 lis

論文名 1 Title	The impact of mitochondrial quality control by Sirtuins on the treatment of type 2 diabetes and diabetic kidney disease(in revise)					
掲載誌名 Published journal	BBA - Molecular Basis of Disease					
	年 月	巻（号）	頁 ～ 頁	言語 Language	English	
第1著者名 First author	Jing Xu	第2著者名 Second author	Munehiro Kitada	第3著者名 Third author	Daisuke Koya	
その他著者名 Other authors						
論文名 2 Title						
掲載誌名 Published journal						
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Review

The impact of mitochondrial quality control by Sirtuins on the treatment of type 2 diabetes and diabetic kidney disease

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ABSTRACT

The incidence of type 2 diabetes mellitus (T2DM) and diabetic kidney disease (DKD) has significantly increased worldwide in recent decades, and improved treatments for T2DM and DKD are urgently needed. The pathogenesis of aging-related disorders, such as T2DM and DKD, involves multiple mechanisms, including inflammation, autophagy impairment, and oxidative stress, which are closely associated with mitochondrial dysfunction. Therefore, mitochondrial quality control may be a novel therapeutic target for T2DM and DKD. Previous reports have shown that members of the mammalian Sirtuin family, SIRT 1–7, which are recognized as antiaging molecules, play a crucial role in the regulation of mitochondrial function and quality control through the modulation of oxidative stress, inflammation and autophagy. In this review, we summarized the research published in recent years to highlight the role of Sirtuins in mitochondrial quality control as a therapeutic target for T2DM and DKD.

Abbreviations: T2DM, type 2 diabetes mellitus; DKD, diabetic kidney disease; AKI, acute kidney injury; sir2, silent information regulator 2; SIRT, Sirtuins; HFD, high-fat diet; CR, calorie restriction; RSV, resveratrol; ZDRs, Zucker diabetic rats; WFRs, Wistar fatty rats; HUVECs, human umbilical vein endothelial cells; MEF, murine embryonic fibroblasts; hESCs, human embryonic stem cells; EPCs, endothelial progenitor cells; eGFR, estimated glomerular filtration rate; TCA, citric acid cycle; AMPK, AMP activated kinase; p-AMPK, phosphorylated-AMP activated kinase; CaMKKβ, Ca^{2+}/calmodulin-dependent protein kinase kinaseβ; ERK, extracellular signal-regulated kinase; CPS1, carbamoyl phosphate synthetase 1; ECHA, trifunctional enzyme subunit alpha; HMGCS2, 3-hydroxy-3-methylglutaryl-CoA synthase 2; PI3K, phosphoinositide 3-kinase; MCD, malonyl-CoA decarboxylase; PKM2, pyruvate kinase isozyme M2; G6PD, glucose-6-phosphate dehydrogenase; AceCS2, acetyl-CoA synthetase 2; GDH, glutamate dehydrogenase; SDH, succinate dehydrogenase; LDH, lactate dehydrogenase; TPI, triose phosphate isomerase; PFK1, aldolase, and phosphofructokinase-1; PEPCK, phosphoenolpyruvate carboxykinase; SDHA, succinate dehydrogenase subunit A, flavoprotein; IDH2, isocitrate dehydrogenase 2; LCAT, lecithin cholesterol acyltransferase; MCAD, medium-chain acyl-CoA dehydrogenase; VLCAD, very-long-chain acyl-CoA dehydrogenase; CPT1, carnitine palmitoyltransferase; MTCO2, mitochondrially encoded cytochrome C oxidase II; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PKA, protein kinase A; PKB, protein kinase B; PINK1, PTEN induced putative kinase 1; GLUT1, glucose transporter 1; HIF1α, hypoxia inducible factor1α; PDC, pyruvate dehydrogenase complex; GCN5, nonrepressed protein 5; NAD^+, nicotinamide adenine dinucleotide; ATP, adenosine triphosphate; Mfn1/2, mitofusion1/2; OPA1, optic atrophy 1; Drp1, dynamin-related protein 1; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1α; TFAM, mitochondrial transcription factor A; ETC, subunit of electron transport chain; ANT2, adenine nucleotide translocator2; GABPβ1, GA-binding protein; PFS^{mt}, mitochondrial protein folding stress; AdipoR1, adiponectin receptor 1; PPARα, peroxisome proliferator-activated receptor α; ROS, reactive oxygen species; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2; MnSOD, manganese superoxide dismutase; OXPHOS, oxidative phosphorylation; Prx3, peroxiredoxins 3; Prx5, peroxiredoxins 5; Trx2, thioredoxin 2; TR2, thioredoxin reductase 2; UCP-2, uncoupling protein 2; FOXO, forkhead box O; Ace-FOXO1, acetylated-forkhead box O 1; Nrf2, NF-E2-related factor 2; ARE, antioxidant response element; NF-κB, nuclear factor-κ B; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein 1; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; TLR, Toll-like receptor; AGEs, advanced glycation end products; EMT, epithelial-mesenchymal transition; TGFβ, transforming growth factor β; STAT3, signal transducer and activator of transcription 3; HO-1, heme oxygenase-1; p66Shc, 66-kDa Src homology 2 domain-containing protein; LC3, microtubule-associated protein light chain 3; Atg5/7, autophagy related 5/7; mTORC1, rapamycin complex 1; BNIP3, BCL2/adenovirus E1B 19 kDa protein-interacting protein 3; PARK2, parkin 2; MCCC, methylcrotonyl-CoA carboxylase complex; Acyl-MCCC, acylated-methylcrotonyl-CoA carboxylase complex; PDX1, pancreatic and duodenal homeobox 1; ERR, estrogen-related receptor; RNAP II, RNA polymerase II; IGF-1, insulin like growth factor-1; eNOS, nitric oxide synthase

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1. Introduction

The prevalence of type 2 diabetes mellitus (T2DM) has been gradually increasing worldwide in recent decades [1]. Between 2001 and 2009, the prevalence of T2DM increased from 3.4% to 4.6% in the United State [2] and from 9.3% in 2010 to 10.9% in 2013 in China [3]. Accompanying with the increasing prevalence, the incidence of its chronic complications, such as diabetic kidney disease (DKD) also increased (from 19.5% in 2010 to 24.3% in 2015 in China) [4]. DKD is considered as the main cause of end-stage renal diseases and an independent risk factor for cardiovascular diseases [5]. All of these disorders bring an enormous burden to the healthcare system worldwide. Although several new drugs such as SGLT2 inhibitors or GLP-1 agonist have been developed to treat T2DM and presented prospective outcomes in recent years [6–11], there is still an urgent need for more effective therapies for T2DM and DKD.

Aging is an inevitable and universal process. It increases oxidative stress and inflammation caused by mitochondrial dysfunction and weakens the responsiveness to intracellular stress, ultimately leads to metabolic dysfunction and the disruption of cellular homeostasis [12]. Inflammation is also considered an important role in the pathogenesis of aging-related diseases [13]. Nuclear factor kappa-B (NF-κB) is the central transcriptional factor involved in inflammation, and it mediates the expression of multiple inflammatory factors, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), [14]. Aging and cellular senescence may accelerate the progression of T2DM and DKD [12], associating with inflammation and mitochondrial dysfunction [15–17]. Therefore, mitochondrial quality control might be a potential target for the treatment of age-related diseases such as T2DM and DKD.

Mitochondrial quality control involves a variety of mechanisms, among which the regulation by Sirtuins is a highlighted direction. The Sirtuin family contains highly conserved nicotinamide adenine dinucleotide (NAD^+)-dependent histone/protein deacetylases and ADP-ribosyltransferases [18,19]. Sirtuins (SIRT1–7) are recognized as antiaging molecules and have been confirmed to participate in multiple cellular processes including the regulation of mitochondrial function, oxidative stress, inflammation and autophagy [20], which is correspondingly related to the pathogenesis of T2DM and DKD.

In this review, we summarized studies published in recent years to highlight the role of Sirtuins in mitochondrial quality control related to the improvement of mitochondrial function/biogenesis/fission and fusion balance, anti-oxidative stress/inflammation and induction of autophagy as a therapeutic target for T2DM and DKD.

2. Mitochondrial dysfunction on the pathogenesis of T2DM and DKD

Mitochondrial dysfunction has been identified to be linked to the pathogenesis of T2DM and DKD. Clinical studies have shown that T2DM patients have fewer mitochondria, lower mitochondrial density and adenosine triphosphate (ATP) production than normal individuals [21,22]. Additionally, a previous study reported that glycolytic enzymes including pyruvate kinase M2 (PKM2) and mitochondrial enzymes including mitochondrially encoded cytochrome C oxidase II (MTCO2) are significantly elevated in glomeruli from individuals with extreme duration of type 1 diabetes (≥50 years) without diabetic nephropathy compared to those with histologic signs of diabetic nephropathy [23]. Moreover, in T2DM patients, these enzymes including PKM2 and MTCO2 are significantly increased in glomeruli of CKD^- individuals, compared to CKD^+ individuals [24]. These data indicate that maintaining mitochondrial function or mitochondrial quality control in the kidney is important for protecting against DKD.

Mitochondrial quality control in cells mainly involves the regulation of redox status, fusion and fission procedures, autophagy/mitophagy and biomolecular repair/biogenesis [15]. The disruption of either of these quality control pathways is a major cause of mitochondrial dysfunction and leads to oxidative stress, inflammation, contributes to the pathogenesis of mitochondrial-related diseases, ranging from inherited diseases to age-related disorders, T2DM and its complications including

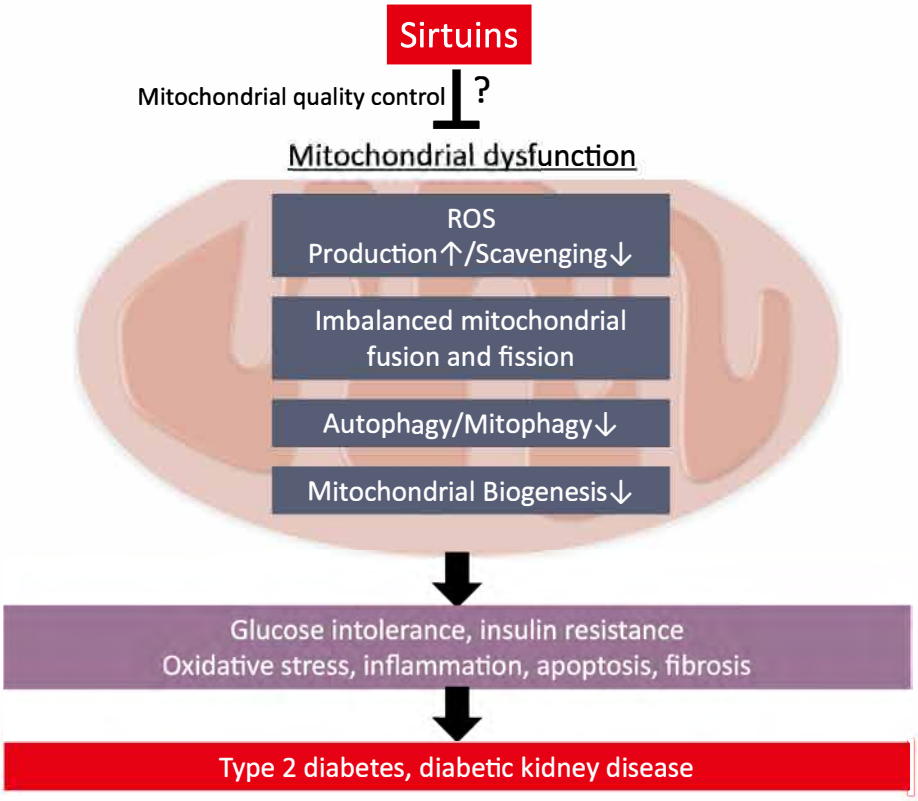

Fig. 1. Mitochondrial dysfunction includes imbalance between reactive oxide species (ROS) production and scavenging, imbalanced fusion and fission, impaired autophagy/mitophagy and reduced mitochondrial biogenesis, which contribute to the pathogenesis for type 2 diabetes and diabetic kidney disease through glucose intolerance, insulin resistance, oxidative stress, inflammation, excess apoptosis and fibrosis in diabetic kidney. Sirtuins may be a potential target for the treatment of these diseases through mitochondrial quality control.

DKD [25,26] (Fig. 1).

2.1. Redox status in mitochondrial

Mitochondria are main source of reactive oxygen species (ROS) [27], and mitochondrial ROS is scavenged by antioxidant enzymes such as superoxide dismutase 2 (SOD2), known as a manganese superoxide dismutase (MnSOD), [28]. Therefore, mitochondrial dysfunction results in the enhancement of oxidative stress by increased production of ROS from injured mitochondria and impairment of SOD2. In diabetic state, mitochondria exhibit increased production of ROS due to impaired electron transport and ROS scavenging, then contribute to the pathogenesis of insulin resistance/diabetes and DKD [16,17,29] (Fig. 1). Additionally, oxidative stress is closely related to inflammation, therefore, mitochondrial quality control is also important for suppression of both oxidative stress and inflammation.

2.2. Balance of fusion and fission

Fusion and fission are crucial to maintaining mitochondrial stability and biological function [15]. The expression of mitochondrial fusion protein (mitofusion2; Mfn2 and optic atrophy1; Opa1) is reduced in skeletal muscles of patients with T2DM [30]. Our previous studies found that mitochondria fusion proteins such as Mfn1/2 and Opa1 in the kidney are inhibited by a high-fat diet (HFD) [31], and fission proteins such as dynamin-related protein 1 (Drp1) are increased in renal cortex of Zucker diabetic rats (ZDRs), a T2DM rat model [32]. Other report demonstrated that a deficiency in Mfn2 leads to increased superoxide and the activation of NF-κB, leading to insulin resistance in rat skeletal muscle cells [33].

2.3. Autophagy and mitophagy

Mitophagy is a selective autophagy that recognizes damaged mitochondria for degradation through fission of mitochondria [34]. Starvation is well known to activate autophagy, while starvation leads to inhibition of mitochondrial fission through protein kinase A (PKA)-induced phosphorylation of Drp1, then results in mitochondrial elongation [35]. Multiple studies have identified the key role of autophagy in the pathogenesis of T2DM and its chronic complications, such as DKD [36,37]. Our previous study also observed that the accumulation of p62/Sqstm1 and abnormal mitochondria are significantly enhanced in the kidneys of Wistar fatty rats (WFRs), a rat model of T2DM, indicating dysregulation of autophagy [38,39]. Thus, regulation of mitochondrial fission/fusion balance and autophagy/mitophagy play a crucial role on mitochondrial quality control to improve T2DM and DKD (Fig. 1).

3. Mammalian Sirtuins family

Sirtuins are derived from silent information regulator 2 (sir2) in research on the cause of aging in yeast [18,19]. Sirtuins are highly conserved from bacteria to mammals. Seven human Sirtuin genes (SIRT1–7) have been identified and divided into four phylogenetic classes, known as classes I–IV [40]. Table 1 lists the Sirtuin family members and their characteristics.

Table 1
Sirtuins family and their characteristics.

Sirtuins	Classification	Enzyme activity	Location
SIRT1	I	Deacetylase	Nucleus and cytoplasm
SIRT2	I	Deacetylase Demyristoylase	Cytoplasm and nucleus
SIRT3	I	Deacetylase	Mitochondria and cytoplasm
SIRT4	II	ADP-ribosyltransferase Deacetylase Lipoamidase	Mitochondria
SIRT5	III	Deacetylase Desuccinylase Demalonylase	Mitochondria
SIRT6	IV	Deacetylase ADP-ribosyltransferase	Nucleus and endoplasmic reticulum
SIRT7	IV	Deacetylase	Nucleus and cytoplasm

3.1. SIRT1

SIRT1 is the most widely studied member of the Sirtuin family. SIRT1 is mainly located in the nucleus and shuttles between the nucleus and cytoplasm under physiological and pathological stress [41]. SIRT1 deacetylates histone, such as H4 lysine 16 (H4-K16Ac), H3 lysine 9 (H3-K9Ac), and H1 lysine 26 (H1-K26Ac) and regulates the activity of multiple transcription factors and proteins via deacetylation, which involves mitochondrial biogenesis, redox homeostasis, inflammation and autophagy [40,42] (Fig. 2).

3.1.1. Role in mitochondrial biogenesis and oxidative stress

Peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) is a key transcriptional coactivator that regulates mitochondrial biogenesis, mitochondrial respiration and redox status through the induction of the oxidative phosphorylation (OXPHOS) genes expression and anti-oxidative enzymes [43,44]. SIRT1 deacetylates PGC-1α to increase mitochondrial biogenesis and mitochondrial fatty acid oxidation in myotubes [45]. Calorie restriction (CR) or fasting can induce PGC-1α deacetylation via SIRT1, which leads to increased mitochondrial biogenesis and the activation of mitochondrial fatty acid oxidation genes in skeletal muscle or white adipose tissue [45]. As a key regulator of nutrient and energy expenditure, AMP-activated kinase (AMPK) enhances SIRT1 activity by increasing cellular NAD^+ levels, resulting in the deacetylation and modulation of the activity of downstream SIRT1 targets [46,47]. Adiponectin, which is an antidiabetic hormone that maintains glucose and fatty acid metabolism, combines with adiponectin receptor 1 (AdipoR1) to induce the expression and activation of Ca^{2+}/calmodulin-dependent protein kinase kinaseβ (CaMKKβ), AMPK, and SIRT1 and to decrease the acetylation of PGC-1α in a Ca^{2+}-dependent manner to regulate mitochondrial biogenesis, which further relieves insulin resistance in skeletal muscle [48]. Additionally, SIRT1 activates peroxisome proliferator-activated receptor α (PPARα), a major regulator of lipid metabolism, via PGC-1α deacetylation to enhance fatty acid oxidation in skeletal muscle [45,48]. Increased PGC-1α activity and expression increases the expression of ROS-detoxifying enzymes, such as SOD2 [44]. SIRT1 regulates the expression of several antioxidant genes in bovine aortic endothelial cells, including SOD2, catalase, peroxiredoxins 3 and 5 (Prx3, Prx5), thioredoxin 2 (Trx2), thioredoxin reductase 2 (TR2), and uncoupling protein 2 (UCP-2) through the formation of a FOXO3a/PGC-1α complex [49]. The beneficial effects of SIRT1 on diabetic renal injuries correlate with the activation of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and antioxidant response element (ARE) (Nrf2/ARE) antioxidative pathway, which leads to overexpression of antioxidative enzymes such as heme oxygenase-1 (HO-1) and superoxide dismutase 1 (SOD1) [50]. Additionally, advanced glycation end products (AGEs) are one of the main causes of DKD. The activation of the Nrf2-ARE pathway by the overexpression of SIRT1 ameliorates mitochondrial oxidative stress, further relieving the toxicity of high glucose to podocytes in db/db mice [51] (Fig. 2A). Epithelial-mesenchymal transition (EMT) plays a pivotal role in the pathogenesis of renal tubulointerstitial fibrosis, which is closely related to the pathogenesis for progression of DKD. Previous report demonstrated that aldosterone-induced EMT is dependent on mitochondrial-derived oxidative stress, and SIRT1 restores aldosterone-induced mitochondrial dysfunction and EMT by upregulating PGC-1α [52]. The 66-kDa Src homology 2 domain-containing protein (p66Shc) is phosphorylated at serine 36 (S36) in response to ROS and

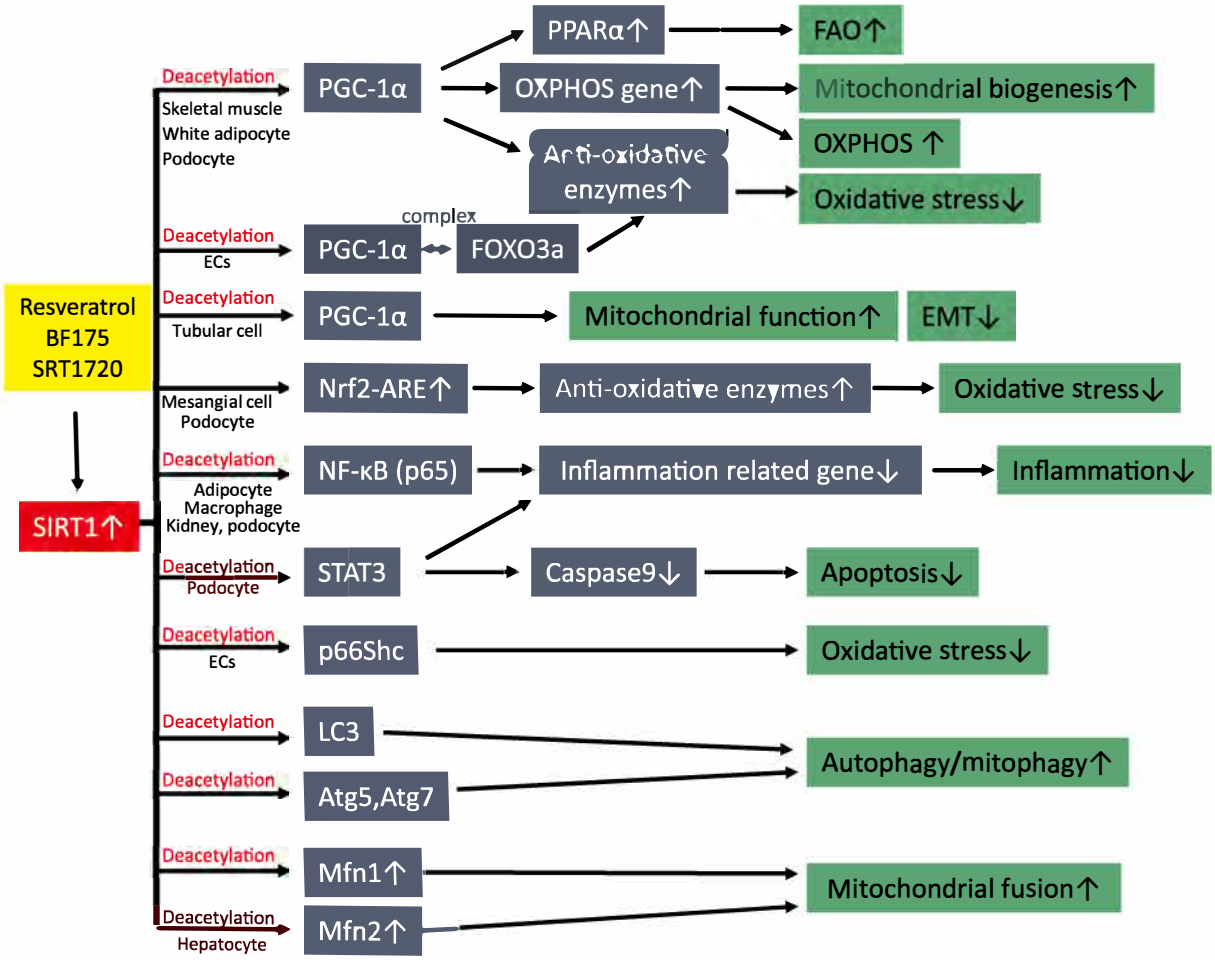

Fig. 2. SIRT1 regulates mitochondrial function related to fatty acid oxidation (FAO), mitochondrial biogenesis, oxidative phosphorylation (OXPHOS), oxidative stress, inflammation, epithelial-mesenchymal transition (EMT), apoptosis, autophagy/mitophagy and mitochondrial fusion, through the multiple mechanism.

translocates to mitochondria, where it produces ROS by oxidizing cytochrome C [53]. SIRT1-mediated deacetylation of p66Shc suppresses vascular oxidative stress and endothelial dysfunction in diabetes [54].

3.1.2. Role in inflammation

Previous studies have demonstrated that SIRT1 deacetylates the RelA/p65 subunit of NF-κB at Lys310 to inhibit its transcription [55]. The activation of SIRT1 inhibits inflammatory pathway through deacetylation of NF-κB (p65) in adipocytes and macrophages to improve glucose tolerance and insulin sensitivity [56,57]. Our previous research also demonstrated that in the proximal tubular cells of WFRs, the expression of inflammation-related genes such as monocyte chemoattractant protein 1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and the acetylated-NF-κB (p65) increased, while CR can alleviate the expression of inflammatory factors by elevating levels of SIRT1, further deacetylating NF-κB [38]. In the podocytes of db/db mice, the deletion of SIRT1 leads to the acetylation of NF-κB (p65) and signal transducer and activator of transcription 3 (STAT3), which results in increased susceptibility to diabetic renal injuries, including inflammation and apoptosis [58] (Fig. 2B).

3.1.3. Role in autophagy

Impaired autophagy is involved in the development of a variety of aging-related diseases [59], especially T2DM and DKD [36]. SIRT1 is considered to be a positive regulator of autophagy, which can deacetylate essential autophagic factors, such as autophagy-related 5 (Atg5), Atg7 and microtubule-associated protein light chain 3 (LC3), leading to the induction of autophagy [60,61]. We previously reported that dietary restriction can ameliorate the impaired autophagy in the kidney of WFRs and can restore SIRT1 levels and degrade p62/Sqstm1 [38]. In addition to the deacetylation effect, the inactivation of SIRT1 also results in the phosphorylation of NF-κB p65, leading to inflammation, the activation of the mechanistic target of rapamycin complex 1 (mTORC1) pathway and the inhibition of AMPK in cultured human monocytes. These results connect autophagy and inflammation together [38]. SIRT1 deacetylates mitochondrial fusion-related proteins, results in mitochondrial quality control. A research reported that SIRT1 deacetylases Mfn1 and up-regulates Mfn1 protein stability, leading to mitochondrial elongation [62]. Additionally, SIRT1 deacetylates Mfn2, leading sequentially to enhancement of autophagy, maintaining mitochondrial quality and cell survival in hepatocytes [63].

3.2. SIRT2

SIRT2 is widely distributed in various tissues and organs and is especially highly expressed in metabolic-related organs such as brain, liver, muscle, adipose, kidney, and pancreas [64]. SIRT2 is located primarily in the cytoplasm and can also be found in the nucleus when cells are in the G2/M transition of the cell cycle and during mitosis [65]. SIRT2 functions mainly as an NAD^+-dependent histone deacetylase [66] and demyristoylase [67]. Itis related to multiple processes, including energy metabolism, inflammation, oxidative stress, mitochondrial function, autophagy, and metabolic process including

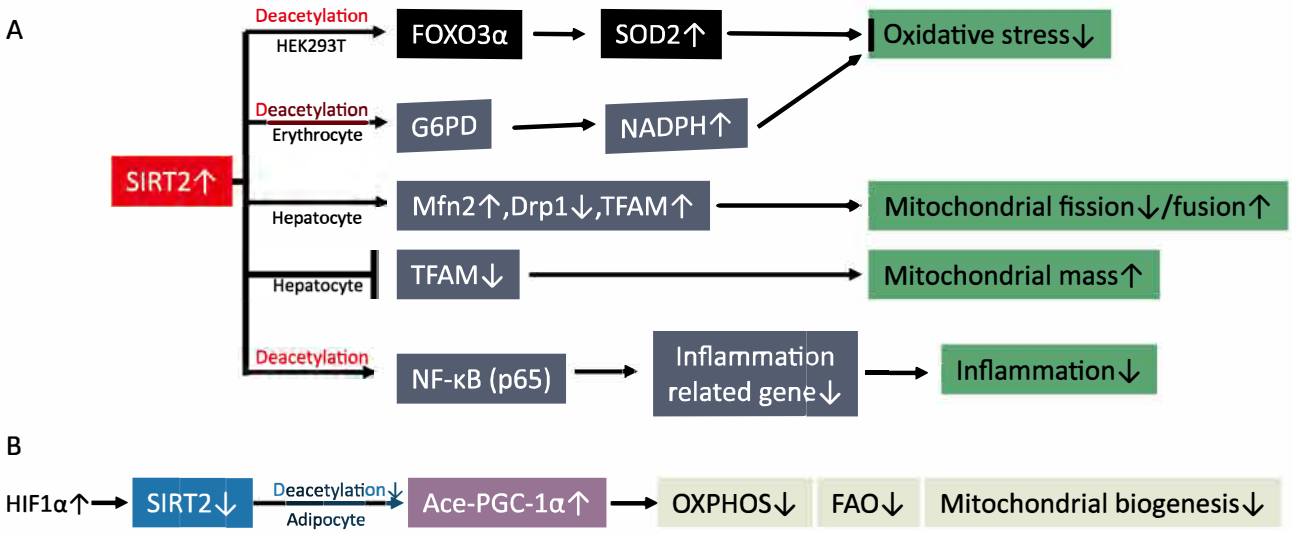

Fig. 3. (A) SIRT2 regulates mitochondrial function related to oxidative stress, inflammation, mitochondrial biogenesis and mitochondrial fission/fusion balance, the multiple mechanism. (B) In adipose tissue, SIRT2 dysfunction due to HIF1α induces increased acetylated PGC-1α, resulting in mitochondrial dysfunction including reduction of fatty acid oxidation (FAD), oxidative phosphorylation (OXPHOS) and mitochondrial biogenesis. In cancer cells, inactivation of SIRT2 induces increased acetylated FOXO1 in cytosol, which binds to Atg7, resulting in induction of autophagy.

T2DM and DKD [64] (Fig. 3). Several studies have shown that SIRT2 is suppressed when energy is excessive and activated when energy is insufficient, indicating that SIRT2 is closely related to intracellular energy utilization. SIRT2 knockout mice exhibit reduced muscle insulin sensitivity, increased liver insulin resistance and increased body weight under HFD conditions [68], indicating that SIRT2 protects against insulin resistance under overnutrition conditions.

3.2.1. Role in mitochondrial biogenesis and oxidative stress

SIRT2 is closely related to improving oxidative stress and reducing the production of ROS in the development of the pathological mechanisms of insulin resistance and T2DM. SIRT2 deacetylates and activates FOXO3α, then activates the transcription of SOD2, thereby further increases intracellular mitochondria-localized SOD2 antioxidant protein levels, reduces ROS production and improving oxidative stress in HEK 293 T cells [69]. Under oxidative stress, SIRT2 deacetylates and activates glucose-6-phosphate dehydrogenase (G6PD), a key enzyme involved in pentose phosphate pathway, which increases the production of NADPH to counteract oxidative stress in erythrocytes [70]. In the above mentioned effects of SIRT2 against oxidative stress, its regulation of mitochondrial quality may play a crucial role. In hepatocytes, SIRT2 increases Mfn2, decreases Drp1 and attenuates the down-regulation of mitochondrial transcription factor A (TFAM), a key mtDNA-associated protein, to increase mitochondrial mass, contributing to the improvement of insulin sensitivity [71]. In adipocytes, the hypoxia induced by excess energy causes hypoxia inducible factor1α (HIF1α) accumulation, which inhibits SIRT2 activity. HIF1α-induced reduction of SIRT2 activity decreases PGC-1α transcriptional activity by increased its acetylation, which results in decrease of the expression of mitochondrial genes, thereby hindering the catabolism of fatty acids in mitochondria [72].

3.2.2. Role in inflammation

SIRT2 has some similar functions to SIRT1, such as negatively regulating NF-κB-dependent gene expression by deacetylating p65 Lys 310 [73], and has been shown to participate in the pathogenesis of multiple diseases such as colitis and arthritis by regulating the inflammatory pathway [74,75]. Nevertheless, no study has clearly shown whether SIRT2 participates in the pathological development of T2DM and DKD through the inflammatory pathway.

3.2.3. Role in autophagy

SIRT2 is also involved in the autophagy process. Unlike SIRT1 interacts with FOXO1 in the nucleus, SIRT2 deacetylates acetylated FOXO1 in the cytoplasm. Reduction of SIRT2 activity inhibits the deacetylation of FOXO1, and acetylated FOXO1 interact with Atg7 in the cytosol and induce autophagy in cancer cells [76].

3.3. SIRT3

SIRT3 is mainly located in mitochondria, acting as a NAD^+-dependent deacetylase to regulate mitochondrial protein deacetylation [77] and energy homeostasis [78]. Through its deacetylation effects, SIRT3 is involved in the development of metabolic diseases including T2DM and DKD [79] (Fig. 4). Clinical studies have revealed that SIRT3 activity is decreased in skeletal muscle and pancreatic islets [80] in diabetic patients and that high SIRT3 expression levels are associated with longevity [81]. SIRT3 knockout mice show decreased oxygen consumption, reduced glucose-stimulated insulin secretion, elevated acetylation of mitochondrial proteins and increased oxidative stress [80,82,83].

3.3.1. Role in mitochondrial biogenesis and oxidative stress

SITR3 deacetylates acetyl-CoA synthetase 2 (AceCS2), an important rate-limiting enzyme in the citric acid cycle to participate in glycolysis, and deacetylates glutamate dehydrogenase (GDH), which is responsible for amino acid oxidation in the citric acid cycle [84]. SIRT3 also deacetylates long-chain acyl-CoA dehydrogenase (LCAD), a key enzyme in fatty acid oxidation, resulting in the activation of fatty acid metabolism [85]. SIRT3 is essential for the maintenance of basal ATP levels and mitochondrial electron transport. It deacetylates complex I and complex II, especially the succinate dehydrogenase flavoprotein (SDHA) subunit of electron transport chain (ETC), to increase their activity, further elevating mitochondrial oxidative phosphorylation [82,86,87]. The overexpression of SIRT3 deacetylates ATP synthase and further increases ATP levels [82,86,87].

SIRT3 has been shown to play a central role against mitochondrial oxidative stress through the deacetylation and activation of antioxidant enzymes such as isocitrate dehydrogenase 2 (IDH2) and SOD2 [88–90]. Our previous report also demonstrated that the expression of acetylated-SOD2 and -IDH2 was significantly increased in mitochondria isolated from renal cortex of ZDRs, compared to Zucker lean diabetic

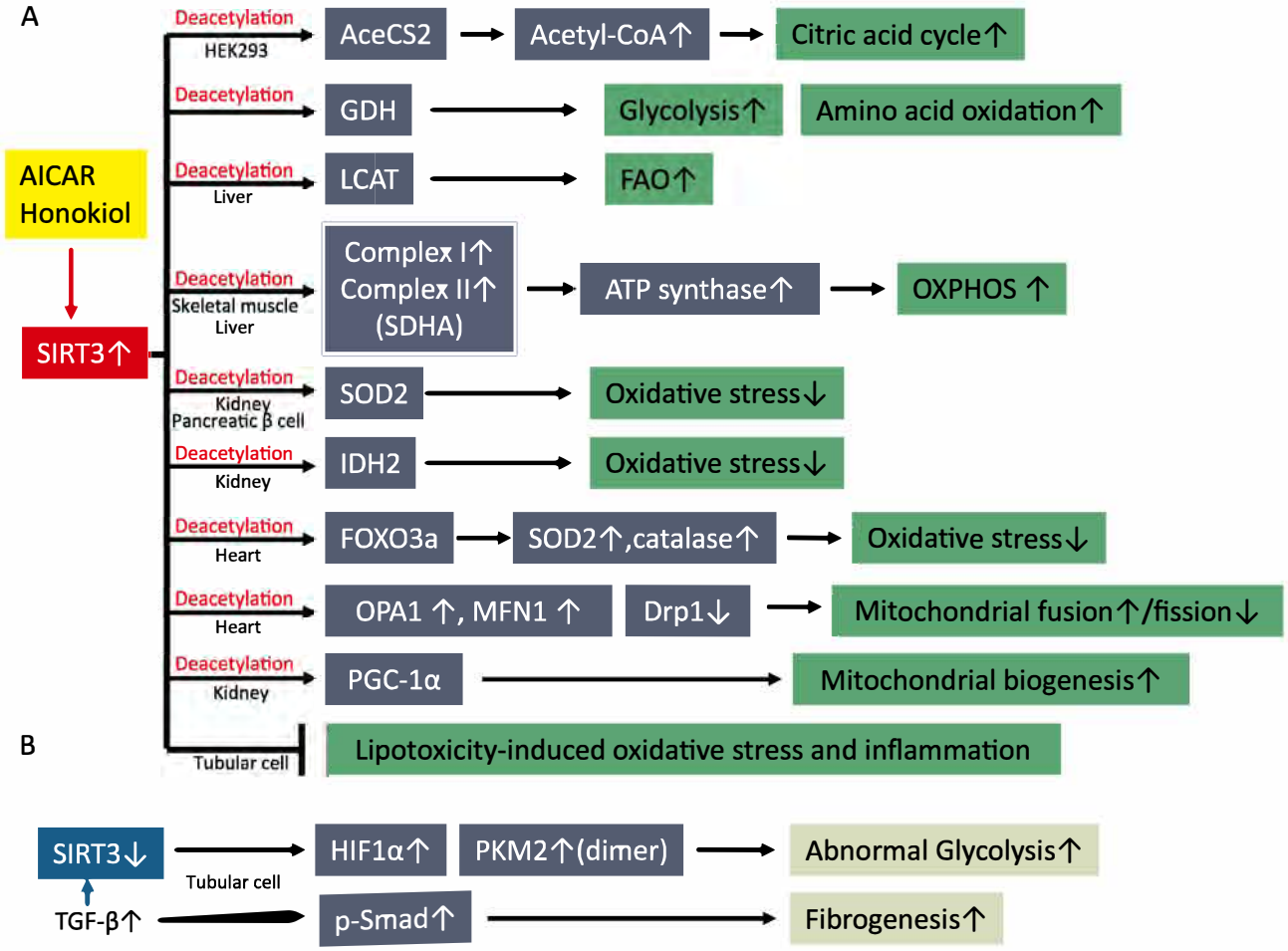

Fig. 4. (A) SIRT3 regulates mitochondrial function related to fatty acid oxidation (FAO), mitochondrial biogenesis, oxidative phosphorylation (OXPHOS), oxidative stress, inflammation, autophagy/mitophagy, and mitochondrial fusion, the multiple mechanism. SITR3 also participates in cellular metabolism including citric acid cycle, glycolysis, acid oxidation. (B) SIRT3 suppression is associated with abnormal glycolysis and fibrogenesis through HIF1α accumulation, Pyruvate kinase M2 (PKM2)(dimer) formation and TGF-β/smad pathway.

rats, which is associated with SIRT3 inactivation in diabetic kidney [32]. Additionally, SIRT3 inhibition increases the acetylation of both SOD2 and p53 protein to aggravate oxidative stress in an acute kidney injury (AKI) rat model [91]. Primary pancreatic islets of SIRT3 knockout mice and pancreatic β cell lines (MIN6) exhibit decreased SIRT3 expression and increased SOD2 acetylation, leading to impaired glucose-stimulated insulin secretion and glucose-stimulated ATP generation, associated with oxidative stress [83]. In addition to the direct deacetylation of SOD2, SIRT3 upregulates the expression SOD2 and catalase by deacetylating FOXO3α to increase its transcriptional activity [92].

SIRT3 is related to mitochondrial fusion and fission processes. Previous research demonstrated that SIRT3 deacetylates and actives mitochondrial fusion proteins such as OPA1 at the lysine 926 and 931 residues and elevates its GTPase activity to regulate mitochondrial dynamics and further protects cardiomyocytes from stress [93]. The AMPK activator, 5-aminoimidazole-4- carboxamide-1-β-D-ribofuranoside (AICAR) can reduce cisplatin-induced AKI and improve renal function via the deacetylase activity of SIRT3. SIRT3 deficiency exacerbates AKI accompanied by the increased expression of Drp1 and the decreased expression of OPA1 and PGC-1α, which leads to a shift in mitochondria dynamics toward fission [94].

3.3.2. Role in inflammation

Currently, there are limited reports on SIRT3 and inflammation. In a rat insulinoma Cell line (INS1 cells), SIRT3 knockdown results in not only impaired insulin secretion but also impaired protective effects of nicotinamide mononucleotide on inflammatory cytokines, such as TNF-α and IL-1β [83]. Another research showed that AGEs decrease SIRT3 expression in endothelial progenitor cells (EPCs) and increase IL-8, which may be involved in the pathogenesis of diabetes-related vascular complications [95]. Additionally, SIRT3 ameliorates lipotoxicity-mediated ROS and inflammation in renal proximal tubular cells [96]. Furthermore, our previous study showed that SIRT3 suppression associated activation of transforming growth factor β (TGFβ)/Smad signaling and renal fibrosis through induction of abnormal glycolysis by modulating the HIF1α accumulation and increase PKM2 dimer formation, leading to abnormal glycolysis and, ultimately, diabetes-associated kidney fibrosis [97].

3.3.3. Role in autophagy

The relationship between SIRT3 and autophagy in different tissues and organs shows different results. Under HFD condition, SIRT3 overexpression causes AMPK inhibition and mTORC1 activation, resulting in autophagy suppression in hepatocytes [98]. However, SIRT3 overexpression can upregulate p-AMPK and downregulate p-mTOR to promote autophagy in AKI model mice [99].

3.4. SIRT4

SIRT4 is considered to be a mitochondrial protein located in the mitochondrial matrix and is widely expressed in multiple organs and

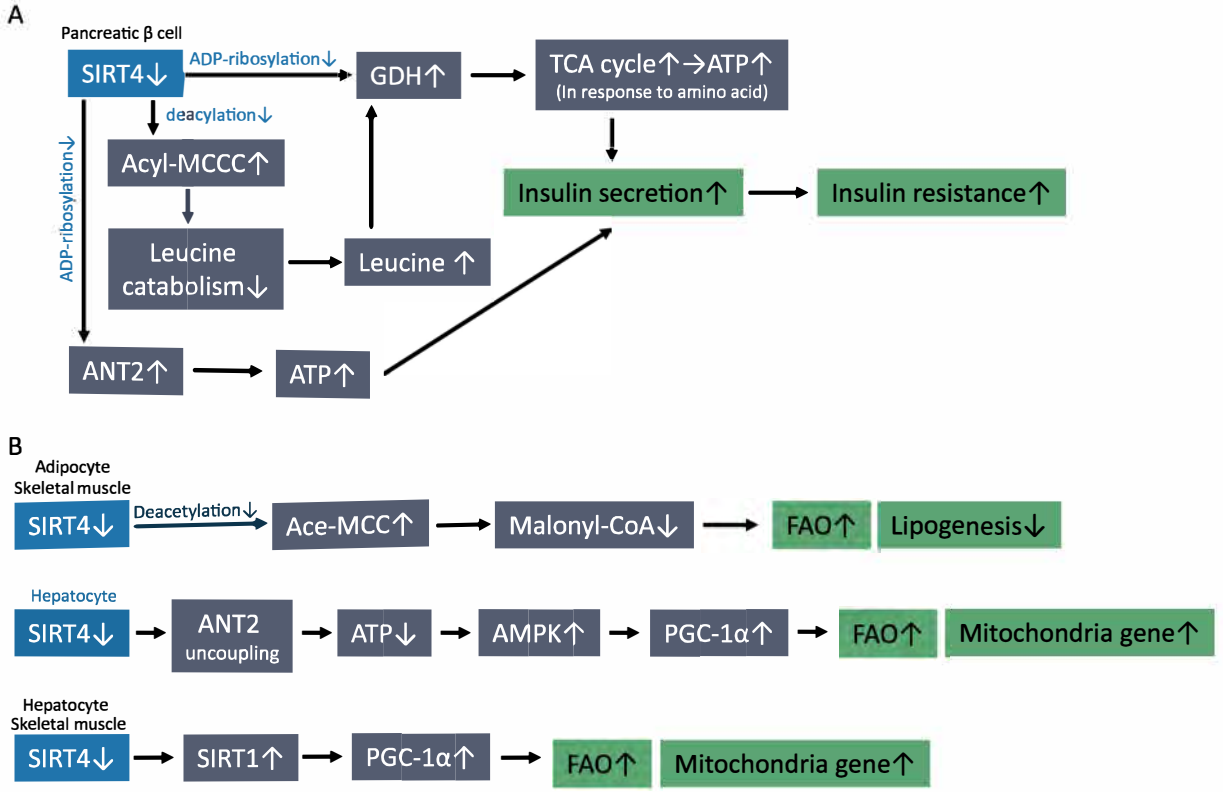

Fig. 5. (A) SIRT4 suppression positively regulates insulin secretion from pancreatic β cell, and chronic elevated insulin secretion progresses insulin resistance. (B) SIRT4 suppression induces the increased fatty acid oxidation (FAO) and mitochondrial gene expression, and reduction of lipogenesis.

tissues of mammals including liver, muscle and kidney [100]. SIRT4 is characterized as a NAD^+-dependent ADP-ribosylase, deacylase and acylase, and is involved in the regulation of metabolism and mitochondrial function [101] (Fig. 5). Previous reports showed that SIRT4 in mitochondria is related to the regulation of insulin secretion from β cell and glucose tolerance. Pancreatic β cells in SIRT4 deficient mice exhibits promotion of insulin secretion, which is associated with GDH activation [101]. GDH catalyzes the conversion of glutamate to α-ketoglutamate, an intermediate of the citric acid cycle (TCA) cycle. Through the utilization of glutamate and the increasing of mitochondrial ATP production, GDH is activated to promote insulin secretion, while SIRT4 suppresses GDH activity by its ADP-ribosylation, resulting in the downregulation of insulin secretion [101]. In SIRT4-depleted INS-1E cells, insulin secretion is markedly increased under high glucose conditions. SIRT4 catalyzes the ADP-ribosylation of adenine nucleotide translocator2 (ANT2), an ATP/ADP translocase that transports ATP into the cytosol and ADP into the mitochondrial matrix, to reduce ATP production, then negatively regulates insulin secretion [102]. Additionally, SIRT4-deficient mice exhibit the elevated basal and stimulated insulin secretion through leucine-induced GDH activation, leading to develop age-related glucose intolerance and insulin resistance [103]. The absence of SIRT4 increases and destabilizes methylcrotonyl-CoA carboxylase complex (MCCC) acylation, leading to decreased leucine oxidation.

3.4.1. Role in mitochondrial biogenesis

SIRT4 may exhibit the opposite functions by decreasing mitochondrial function including fatty acid oxidation, compared to SIRT3. During the fed state, SIRT4 inhibits the activity of malonyl-CoA decarboxylase (MCD) through deacetylation of its enzyme, resulting in an increase in malonyl-CoA. Increased malonyl-CoA promotes lipid synthesis and suppresses fatty acid oxidation by inhibition of carnitine palmitoyltransferase (CPT1), in white adipose tissue and skeletal muscle of mice [104]. In contrast, the loss of SIRT4 can activate AMPK via ANT2 uncoupling with SIRT4 to decrease ATP levels in insulin-producing INS-1E cells [105]. Activated AMPK leads to increased PGC-1α expression, resulting in fatty acid oxidation and mitochondrial genes. Another study confirmed that SIRT4 knockdown in primary mice hepatocytes increases the expression of fatty acid oxidation-related genes such as medium-chain acyl-CoA dehydrogenase (MCAD), CPT1 and PPARα, and mitochondrial genes including PGC-1α. SIRT4-mediated these effects were dependent on SIRT1 [106]. The mRNA levels of hepatic SIRT4 were significantly increased in ob/ob, db/db, and KKAy mice, which have obesity, diabetes and hepatic steatosis [106]. In primary myotubes, SIRT4 knockdown resulted in the increased fatty acid oxidation and cellular oxygen consumption [106].

3.4.2. Role in inflammation

To date, a few studies have confirmed that SIRT4 is involved in the inflammatory pathway and oxidative stress. In human umbilical vein endothelial cells (HUVECs), silencing SIRT4 exacerbates the expression of IL-1β, IL-6 and IL-8, while increasing the nuclear translocation and the transcriptional activity of NF-κB [107]. Further research confirmed that SIRT4 overexpression suppresses the Phosphoinositide 3-kinase (PI3K)/Akt/NF-kB pathway and improves oxidized LDL-induced endothelial injury in HUVECs [108]. SIRT4 reverses high glucose-induced decreases in mitochondrial membrane potential and decreases ROS accumulation and inflammation in mouse cultured podocytes [109]. Additionally, a clinical study showed that compared with healthy individuals, T2DM patients have much lower SIRT4 mRNA levels in granulocytes and monocytes [110].

3.5. SIRT5

SIRT5 is another mitochondrial protein member in the sirtuin family and is expressed broadly in multiple organs, especially in the brain,

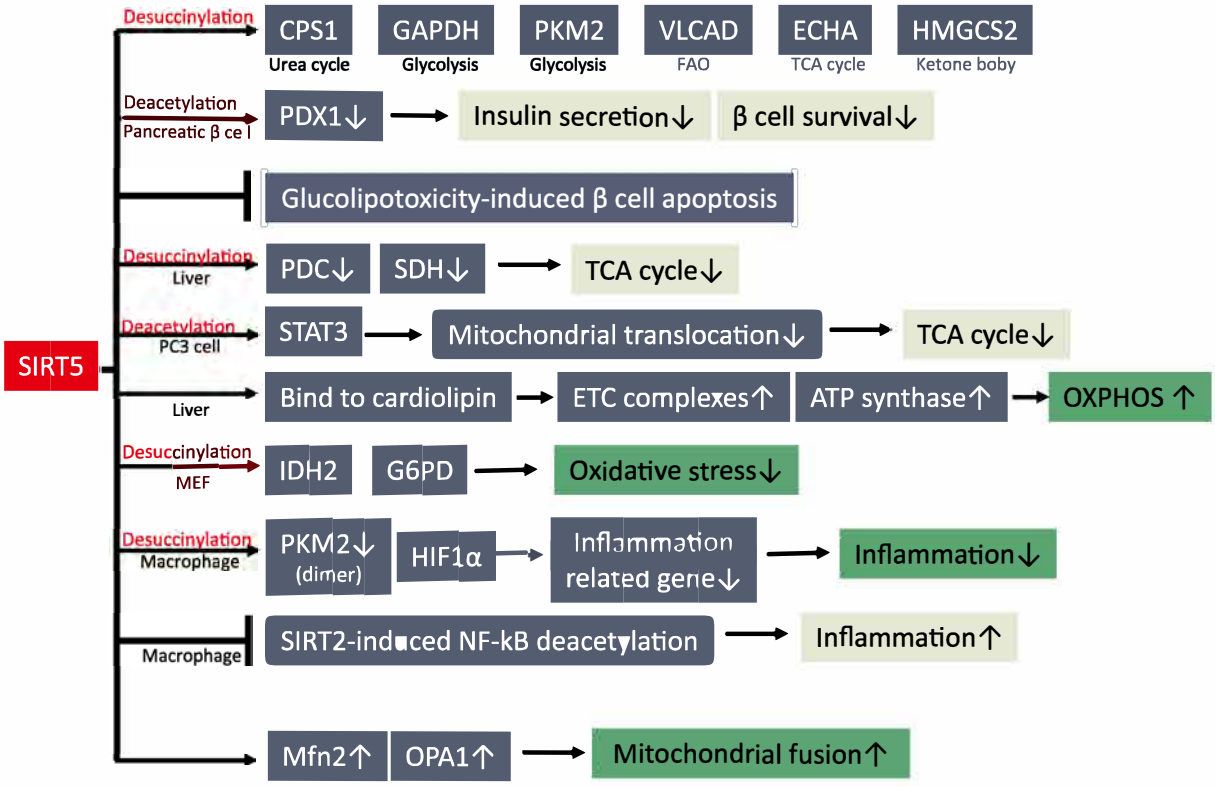

Fig. 6. SIRT5 participates in metabolism including urea cycle, glycolysis, fatty acid oxidation (FAO), TCA cycle and ketone body production. SIRT5 also regulates pancreatic β cell survival, mitochondrial function, oxidative stress and autophagy.

heart, kidney and skeletal muscle [111,112]. SIRT5 participates in the regulation of metabolism and mitochondrial function through multiple mechanisms (Fig. 6). Previous studies showed that SIRT5 is a NAD^+-dependent deacetylase activating carbamoyl phosphate synthetase 1 (CPS1), a critical enzyme for detoxification of excess ammonia, to regulate the urea cycle [112,113]. Other posttranslational modifications of SIRT5 also include malonylation or succinylation on lysine residues in the enzymes associated with glycolysis, fatty acid oxidation and ketone production such as glyceraldehyde 3-phosphate dehydrogenase (GAPDH), PKM2, very long-chain acyl-CoA dehydrogenase (VLCAD), trifunctional enzyme subunit alpha (ECHA) and 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) [113–119]. These findings indicate that SIRT5 may participate in metabolic pathway. Nevertheless, although a global increase in hypersuccinylated proteins and elevated serum ammonia under fasting conditions are observed in SIRT5 knockout mice, no overt metabolic disorders under either chow or HFD conditions are observed [120]. Similarly, despite leading to widespread decreases in protein acetylation, the overexpression of SIRT5 does not have significant effects on mitochondrial or cellular metabolism in mice [121]. In contrast, subsequent research showed that SIRT5 overexpression in ob/ob mice resulted in decreased malonylation and succinylation, leading to improved cellular glycolysis, suppressed gluconeogenesis, enhanced fatty acid oxidation, and attenuated hepatic steatosis [122]. Role of SIRT5 on insulin secretion and pancreatic β cell survival is also controversial. A research demonstrated that SIRT5 negatively regulates pancreatic and duodenal homeobox 1 (PDX1), which is a regulator of insulin gene expression and pancreatic β cell survival, through its deacetylase activity, despite SIRT5 having weak deacetylase activity [123]. Interestingly, SIRT5 mRNA levels were significantly upregulated in plasma of patients with T2DM. However, another research showed that SIRT5 protects pancreatic β cells against glucolipotoxicity-induced apoptosis and decrease in insulin secretion [124].

3.5.1. *Role in mitochondrial biogenesis*

SIRT5 is involved in the regulation of mitochondrial function and oxidative stress. SIRT5 desuccinylates and suppresses activities of pyruvate dehydrogenase complex (PDC) and succinate dehydrogenase (SDH), resulting in reduction of TCA cycle activity [125]. SIRT5 also deacetylates STAT3 and inhibits its mitochondrial translocation, where intern decrease in TCA cycle activity [126]. In contrast, a study showed that SIRT5 binds to cardiolipin and desuccinylates inner mitochondrial membrane proteins including multiple subunits of four ETC complexes and ATP synthase, leading to promote respiratory chain function [119]. On the role of SIRT5 for the regulation of redox status, silencing SIRT5 inhibits IDH2 and G6PD desuccinylation, decreasing NADPH production and impairing the process of scavenging ROS, which leads to increasing cellular oxidative stress in murine embryonic fibroblasts (MEF) [127]. In mouse primary hepatocytes, the overexpression of SIRT5 increased ATP synthesis and oxygen consumption in a dose-dependent manner. SIRT5 is positively regulated by PGC-1α in a PPARα- and estrogen-related receptor (ERR)α-dependent manner; in contrast, interestingly, SIRT5 is negatively regulated by the AMPK activator metformin, which is the most widely used oral medication for T2DM [128].

3.5.2. *Role in inflammation*

The mechanism of the involvement of SIRT5 in inflammation is limited. Hypersuccinylation of PKM2 due to SIRT5 deficiency inhibits its enzymatic by promoting its tetramer-to-dimer transition, leads to promote to entry into nucleus, where a complex of PKM2-HIF1α is formed at the promoter of IL-1β gene in LPS-stimulated macrophages [129]. However, considering that the NAD^+/NADH ratio is associated with inflammation, the decreased levels of NAD^+ were related to the increased expression of SIRT2 and the decreased expression of SIRT5 in endotoxin-tolerant macrophages [130]. SIRT5 deficiency decreased the Toll-like receptor (TLR)-induced expression of inflammatory cytokines, such as IL-6. Competing with SIRT2, which deacetylates NF-κB p65 to relieve inflammation, SIRT5 enhances the acetylation of p65 in a

deacetylase activity-independent manner, which consequently leads to the activation of the NF-κB pathway and its downstream cytokines, such as IL-6, TNF-α and MCP-1 [130].

3.5.3. Role in autophagy

SIRT5 silencing results in the increased succinylation of glutaminase, a key enzyme that transforms glutamine into glutamate to produce ammonia in mitochondria. In this process, autophagy and mitophagy increased the expression of the autophagy markers LC3 paralogs, the mitophagy marker BCL2 Interacting Protein3 (BNIP3) and the mitophagy pathway PINK1-PARK2 in human breast cancer cell lines MDA-MB-231 and mouse myoblast C2C12 [131]. Moreover, in SIRT5-overexpressing cells, the level of mitochondrial fusion markers such as Mfn2 and OPA1 increased, which indicates the relationship between SIRT5 and autophagy via mitochondrial quality control [131].

3.6. SIRT6

SIRT6 is mainly located in the nucleus and functions as a nuclear ADP-ribosyltransferase [132] and NAD^+-dependent deacetylase [20]. SIRT6 has been identified to be involved in a variety of metabolic processes [133–138], lifespan [139,140], inflammation [141–143], DNA damage repair [144,145] and circadian rhythm [146]. SIRT6 is involved in the regulation of metabolism and mitochondrial function through multiple mechanisms (Fig. 7). Role of SIRT6 on glucose homeostasis which is associated with the pathogenesis for T2DM has been showed by several SIRT6 gene altered animals. Whole body SIRT6-deficient mice develop multiple metabolic defects, such as lower insulin like growth factor-1 (IGF-1) levels and severe hypoglycemia, eventually dying at approximately 4 weeks [140]. Liver-specific SIRT6-deficient mice are characterized by increased glycolysis and triglyceride synthesis and reduced β-oxidation, which ultimately leads to fatty liver [133]. Muscle-specific SIRT6-deficient mice show impaired glucose tolerance, insulin resistance, attenuated whole body energy expenditure, and weakened exercise performance [147]. Pancreatic β cell specific SIRT6 knockout mice have lower ATP levels and mitochondrial complex levels in islets and glucose intolerance [137]. The myeloid specific SIRT6 knockout mice fed-HFD exhibited greater increases in body weight, fasting blood glucose and insulin levels, hepatic steatosis, glucose intolerance, and insulin resistance, compared to their wild-type littermates [148]. These findings indicate that SIRT6 may be a potential target involved in the pathogenesis of T2DM.

3.6.1. Role in glucose metabolism

Previous studies have confirmed that SIRT6 plays a pivotal role in the regulation of glycolysis and glycogen synthesis. SIRT6-deficient cells such as muscle cells and ES cells exhibit increased H3K9 acetylation in the promoters of glycolytic genes such as lactate dehydrogenase (LDH), triose phosphate isomerase (TPI), glucose transporter 1 (GLUT1), aldolase, and phosphofructokinase-1 (PFK1), accompanied by increased HIF1α transcriptional activity, leading to the upregulation of glycolysis and diminished mitochondrial respiration [134]. Additionally, tumor suppressor p53 directly activates SIRT6, which deacetylates FOXO1 that in turn reduces the interaction of FOXO1 and its downstream gluconeogenesis gene, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphate (G6P) [149]. Conversely, SIRT6 induces PGC-1α acetylation by enhancing the activity of general control nonrepressed protein 5 (GCN5), which leads to decreases in gluconeogenesis genes, such as G6P and PEPCK, and then suppresses hepatic gluconeogenesis [135].

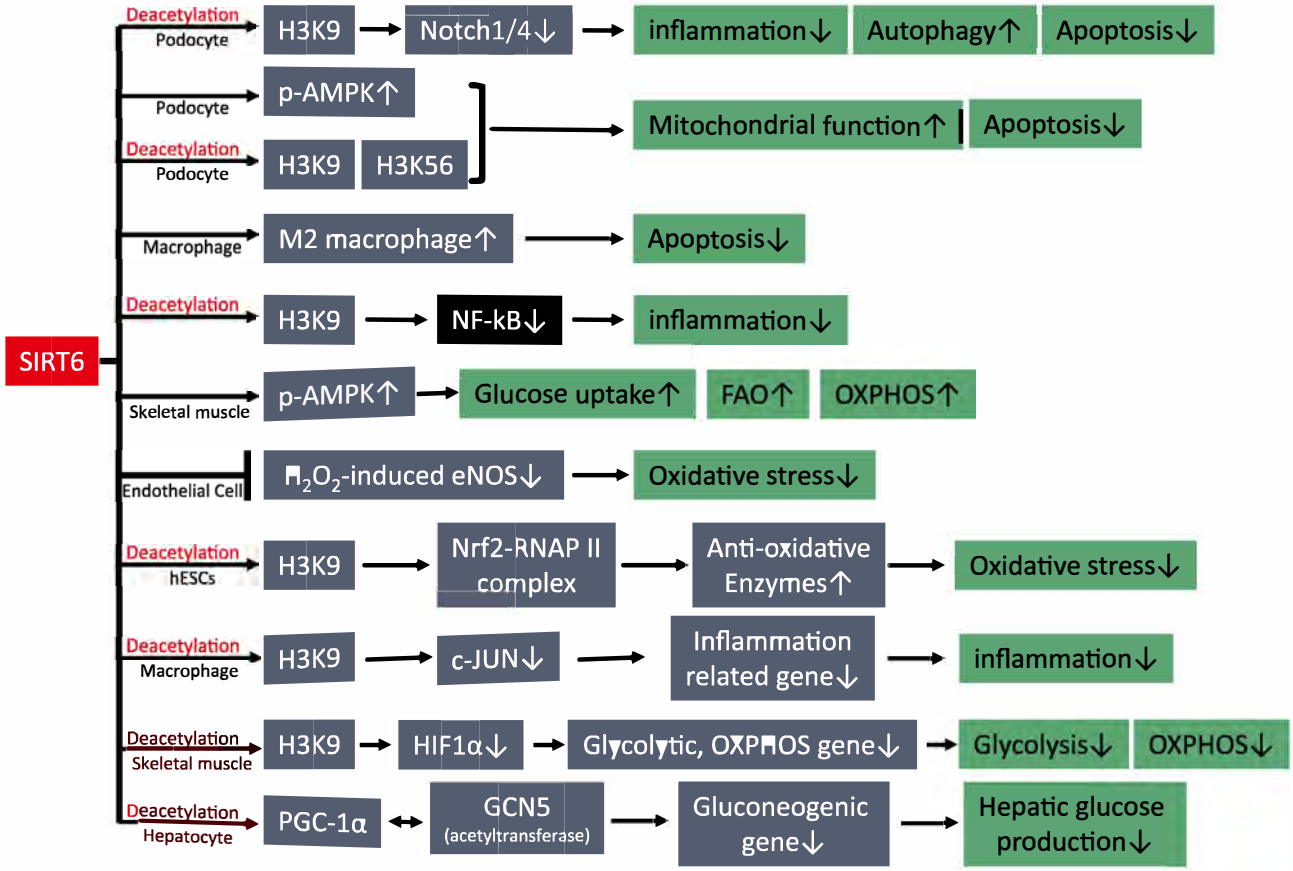

Fig. 7. SIRT6 regulates mitochondrial function related to fatty acid oxidation (FAO), oxidative phosphorylation (OXPHOS), oxidative stress, inflammation, autophagy/mitophagy and apoptosis, the multiple mechanism, and participates in the regulation of glycolysis and hepatic glucose production as well.

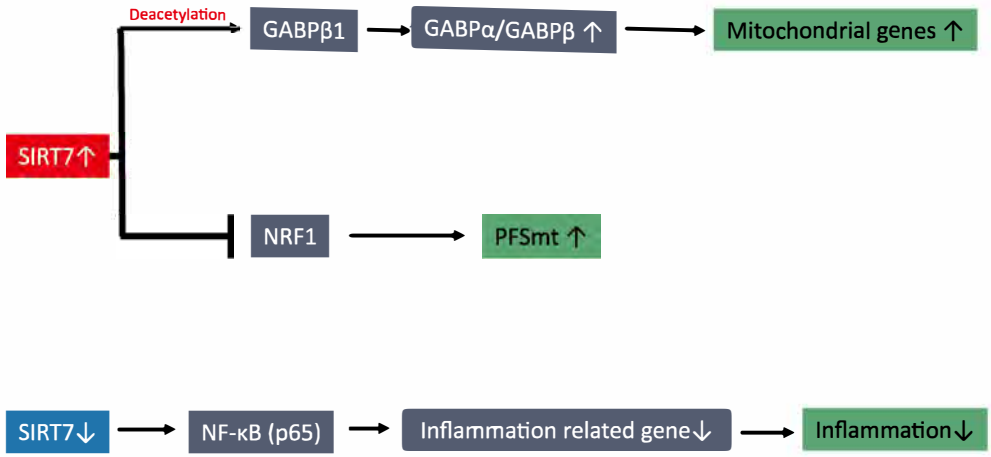

Fig. 8. SIRT7 enhances the expression of mitochondrial genes and ameliorates mitochondrial protein folding stress (PFSmt).

3.6.2. Role in mitochondrial biogenesis

SIRT6 was also shown to be connected to mitochondrial function and oxidative stress. Glucose-stimulated insulin secretion and ATP production are decreased in SIRT6-deficient MIN6 cells, which is related to mitochondrial damage [137]. The deletion of SIRT6 in muscle decreased the expression of genes involved in glucose and lipid uptake, fatty acid oxidation, and mitochondrial OXPHOS because of the lower AMPK phosphorylation [147]. SIRT6 overexpression reduces ROS levels and relieves oxidative stress in glioma cells [150]. SIRT6-deficient human embryonic stem cells (hESCs) exhibit elevated ROS levels, leading to oxidative stress. SIRT6 regulates the cellular redox homeostasis by co-activating Nrf2 antioxidant pathway. SIRT6 associates with Nrf2 and deacetylates H3K56 at the promoter of Nrf2 target genes such as HO-1, which is required for the recruitment of RNA polymerase II complex and subsequent transcriptional activation of Nrf2 and then restoring the oxidative damage caused by SIRT6 deficiency in hESCs [151]. Additionally, SIRT6 overexpression in cultured HVECs attenuates the decreased endothelial nitric oxide synthase (eNOS) level induced by hydrogen oxide (H_2O_2) [152].

3.6.3. Role in inflammation

SIRT6 has been confirmed to be involved in inflammation. It negatively regulates NF-κB signaling by deacetylating H3K9 at chromatin, leading to suppression of inflammation [141]. A clinical study showed that compared with nondiabetic individuals, T2DM patients have decreased SIRT6 expression in carotid plaque obtained from individuals undergoing carotid endarterectomy, which is related to oxidative stress and inflammation [153]. SIRT6-deficient macrophages from Sirt6 knockout mice showed hyperacetylation of H3K9 and increased occupancy of c-JUN in the promoter of inflammatory-related genes, leading to the elevation of their gene expression [142].

3.6.4. Role in DKD

SIRT6 was also found to be involved in the pathogenesis of DKD. The expression of SIRT6 evaluated by immunohistochemistry staining was markedly reduced in renal biopsies from patients with diabetic nephropathy, compared to normal subjects, diabetic patients without nephropathy and patients with other renal diseases such as IgA nephropathy and membranous nephropathy [154]. The mRNA levels of SIRT6 were positively correlated with estimated glomerular filtration rate (eGFR) and negatively correlated with proteinuria [154]. As the mechanism by which SIRT6 protect against diabetes-induced renal injuries, particularly podocyte injury, SIRT6 inhibits Notch1 and Notch4 transcription by deacetylating H3K9 in podocytes, leading to reduction of inflammation, apoptosis and induction of autophagy [154]. Additionally, other report showed that SIRT6 overexpression attenuates high glucose-induced mitochondrial dysfunction in podocytes through H3K9 and H3K56 deacetylation and AMPK activation to maintain mitochondrial function and protect from apoptosis [155]. Furthermore, the overexpression of SIRT6 in macrophages protected podocytes against high-glucose-induced injury such as apoptosis through promotion of the macrophage M2 transformation [156].

3.7. SIRT7

Similar to SIRT1, SIRT7 is located throughout the nucleus and can be found in nucleoplasm, especially in the liver. SIRT7 is the least well-understood member of the sirtuin family [157,158]. An initial study confirmed that SIRT7 interacts with RNA polymerase I and positively regulates its transcriptional activity to maintain cell viability [157]. SIRT7 also functions as an NAD^+-dependent deacetylase to participate in multiple cellular processes, such as DNA repair, cell survival, aging and cancer [158–161]. Numerous studies have shown that SIRT7 is involved in lipid and energy metabolism, which illuminates its potential connection to aging-related diseases such as T2DM, even though these studies are contradictory and controversial [158,159,162,163]. One research showed that SIRT7 knockout mice have a shorter lifespan, heart hypertrophy and inflammatory cardiomyopathy [159]. In contrast, another research showed that SIRT7 knockout mice are resistant to HFD-induced fatty liver, obesity and glucose intolerance [162]. Additionally, SIRT7 knockout mice ameliorates cisplatin-induced AKI and inflammation through reduction of nuclear translocation of NF-κB(p65) and suppressing the expression of TNF-α [164]. However, role of SIRT7 on the pathogenesis for DKD still remains unknown (Fig. 8).

3.7.1. Role in mitochondrial biogenesis

SIRT7 plays important roles in the regulation of mitochondrial function. SIRT7 deacetylates lysine residues located in the hetero- and homodimerization domains of GA-binding protein β1 (GABPβ1), a key regulator of nuclear-encoded mitochondrial genes, which induces the formation of the active GABPα/GABPβ complex and enhances the expression of mitochondrial genes. SIRT7-deficient mice show multisystemic mitochondrial dysfunction, such as increased blood lactate levels, plasma triglycerides and free fatty acids, cardiac dysfunction, and age-related hearing loss, while SIRT7 overexpression rescues these mitochondrial functional defects [165]. Additionally, SIRT7 ameliorates mitochondrial protein folding stress (PFS^{mt}) by suppressing NRF1 activity and reducing the expression of the mitochondrial translation machinery [166].

Table 2
The direct effects of Sirtuins in T2DM and DKD.

Sirtuins	Enzyme activity	Substrates	Effect for pathophysiology of T2DM and DKD	Activators
SIRT1	Deacetylase	PGC-1α	Mitochondrial biogenesis↑ Oxidative stress↓	CR Resveratrol BF175
		Nrf2-ARE	EMT↓	SRT1720
		NF-κB (p65)	Oxidative stress↓	
		STAT3	Inflammation↓	
		LC3,Atg5,Atg7	Apoptosis↓	
		Mfn1, Mfn2	Autophagy/ mitophagy↑ Mitochondrial fusion↑	
SIRT2	Deacetylase	FOXO3α	Oxidative stress↓	–
		G6PD	Oxidative stress↓	
		Mfn2,Drp1,TFAM	Mitochondrial fission↓/fusion↑	
		NF-κB (p65)	Inflammation↓	
		PGC-1α	Mitochondrial biogenesis↑	
SIRT3	Deacetylase	SOD2	Oxidative stress↓	AICAR Honokiol
		IDH2	Oxidative stress↓	
		FOXO3α,catalase	Oxidative stress↓	
		OPA1,MFN1 Drp1	Mitochondrial fission↓/fusion↑	
		PGC-1α	Mitochondrial biogenesis↑	
SIRT6	Deacetylase	Nortch1/4	Inflammation↓, autophagy↑, apoptosis↓	–
		AMPK	Mitochondrial biogenesis↑, apoptosis↓	
		NF-κB, c-JUN	Inflammation↓	
		eNOS	Oxidative stress↓	
		Nrf2-RNAP II complex	Oxidative stress↓	
		HIF1α	Glycolysis↓	
		PGC-1α,GCN5	Hepatic glucose production↓	

4. Activators of Sirtuins in T2DM and DKD

Based on the role of the Sirtuins family mentioned above, especially the role of SIRT1, 3 and SIRT6 in the pathogenesis of T2DM and DKD, activators of sirtuins have been investigating as potential targets for ameliorating T2DM and DKD. Some of them played positive roles in improving mitochondrial function, inhibiting oxidative stress and inflammation.

Resveratrol (RSV) is the most well-known compound for stimulating sirtuins [167]. In a clinical study, RSV increased insulin sensitivity via Akt/protein kinase B (PKB) pathway, then reduced oxidative stress in T2DM patients [168]. It can increase the number of mitochondria in the muscle of KKAy mice by deacetylation of PGC-1α, protecting against diet-induced obesity and insulin resistance [169]. It can also reduce the oxidative damage and apoptosis of podocytes induced by high-glucose stimulation via SIRT1/PGC-1α-mediated mitochondrial protection [170]. In liver of old mice, RSV can reduce the expression of TNF -α, Il-1β , which are increasing during aging [171]. In subsequent studies, more efficient activators were found. SRT1720 is a small molecule activator of SIRT1 that are structurally unrelated to, and 1000-fold more potent than RSV. It can improve insulin sensitivity, lower plasma glucose, and increase mitochondrial capacity in adipose tissue, skeletal muscle and liver of Zucker fa/fa rats [172]. SRT1720 deacetylates PGC-1α to improve mitochondrial biogenesis and NF-κB to inhibit inflammatory pathway in vivo and in vitro [173]. It activates AMPK in a SIRT1-independent manner to increases mitochondrial function in skeletal muscle [174] and attenuates renal fibrosis by inhibiting oxidative stress [175]. BF175 is another new potent, selective agonist of SIRT1. It can protect podocytes from high glucose-induced injury by improving the mitochondrial function and homeostasis via PGC-1α activation in a SIRT1-dependent manner [176] (Fig. 2, Table 2).

As an activator of AMPK, AICAR can reduce cisplatin-induced AKI and improve renal function via the deacetylase activity of SIRT3 [94]. Honokiol is a natural biphenolic compound with anti-inflammatory, anti-oxidative effects [177]. Previous studies demonstrated that as an activator of SIRT3, honokiol increases SIRT3 activity to deacetylate mitochondrial MnSOD in a dose dependent manner to reduce ROS production in cardiomyocytes [177]. Further research showed that honokiol increased expression of MFN1 and OPA1 to maintain the mitochondrial fusion dynamics in cardiomyocytes [178] (Fig. 4, Table2).

5. Conclusion

Existing studies elucidate the role of mitochondrial function in the pathogenesis of T2DM and DKD, showing that the regulation of mitochondrial oxidative stress, biogenesis, mitophagy, fusion and fission processes and other potential mechanisms is involved. As described above, Sirtuins have been confirmed by numerous studies to participate in the regulation of mitochondrial quality control through multiple mechanisms. In particular, SIRT1, 2, 3 and 6 are closely involved in the pathogenesis for T2DM and DKD, we speculate that it may be closely related to their deacetylation effects; therefore, they are considered to be potential targets to relieve insulin resistance, T2DM and DKD. However, there is little direct evidence that SIRT4, 5 and 7 is involved in the pathogenesis for T2DM and DKD. Given its effects on metabolism, and although there are still some contradictions on the physiological role on metabolism and mitochondrial regulation, SIRT4, 5 and 7 is hypothesized to play a role in the pathogenesis for T2DM and DKD.

Transparency document

The Transparency document associated this article can be found, in online version.

Declaration of competing interest

Boehringer Ingelheim, Mitsubishi Tanabe Pharma, Kyowa Hakko Kirin, Taisho Toyama Pharmaceutical Co. and Ono Pharmaceutical Co. contributed to establishing the Division of Anticipatory Molecular Food Science and Technology. The authors declare that there is no duality of interest associated with this manuscript.

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成績状況	優　良　可　不可 学業成績係数=　優	取得単位数 取得単位数 22 単位 ／取得すべき単位総数 22 単位
学生本人が行った研究の概要	GIP は、腸管内分泌 K 細胞から分泌される消化管ホルモンである。GIP はインスリン分泌促進作用に加えて高脂肪食摂取下の肥満やインスリン抵抗性形成に強く関与する。GIP は、長鎖脂肪酸トリグリセリド (LCT) で構成される脂肪の摂取によって過分泌するが、GIP 過分泌抑制は高脂肪食摂取化の肥満やインスリン抵抗性は軽減する。盧氏は、栄養素として中鎖脂肪酸トリグリセリド（MCT）に注目し、長期 MCT 摂取が GIP 分泌や GIP 産生 K 細胞の及ぼす影響について検討してきた。野生型マウスにコントロール食、LCT 食、MCT 食を 3 か月にわたって摂取すると、MCT 食摂取マウスでは LCT 食摂取マウスに比較して体重が低下するにもかかわらず、経口糖負荷後の GIP が LCT に比較して同程度に分泌すること、経口コーンオイル摂取後の GIP 分泌が亢進することを明らかにした。以上から、長期の MCT 摂取は栄養素に対する K 細胞からの GIP 分泌を高める可能性が示唆された。	
総合評価	【良かった点】 研究態度は非常に良好で、他の大学院生とのコミュニケーション能力も高く、実験習得が非常に早い。研究の中で生じる問題の抽出も的確に行える上、問題解決のための研究立案も迅速に行う能力も有している。 【改善すべき点】 研究に熱心になるあまり、研究時間が長時間になることがある。適切な休養も必要と考える。 【今後の展望】 長期の MCT 摂取が栄養素に対する K 細胞からの GIP 分泌を高める機序について、当教室で作製した GIP レポーターマウスを用いて腸管内の K 細胞数や K 細胞内の遺伝子発現を評価する予定である。	
学位取得見込	現在から 2 年以内に上記の評価を行い、3 年以内に学位取得のための論文作成を目指している。	

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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

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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)

高脂肪食を特徴とする食の欧米化を背景として、軽微な肥満であっても糖尿病を発症する患者が本邦で急増している。「高脂肪食摂取による肥満・インスリン抵抗性増大」の機序解明と対策が喫緊の課題である。食事に含まれる「脂肪」は、炭素鎖 (C)14以上の長鎖脂肪酸 (long-chain fatty acid [LCFA]) で構成される長鎖脂肪酸トリグリセリド (long-chain fatty acid triglyceride: LCT) である。

glucose-dependent insulinotropic polypeptide (GIP) は、栄養素の刺激によって腸管内分泌K細胞から分泌され、膵β細胞上のGIP受容体 (GIPR) を介してインスリン分泌を促進する消化管ホルモン (インクレチン) である。特に膵β細胞におけるGIPシグナルは、高脂肪食摂取肥満における高インスリン血症に関与する(1)。そしてGIPRは脂肪組織に存在し、当研究室で作製した脂肪組織特異的GIPR欠損マウスマウスの解析から、in vivoの脂肪組織においてインスリンシグナルが脂肪量増大に重要であること(2)、脂肪組織のGIPシグナルは炎症性サイトカインinterleukin-6 (IL-6) 発現を誘導して高脂肪食摂取下のインスリン抵抗性の形成を助長することが明らかとなった(3)。以上からGIPは脂肪組織への直接的な作用とインスリンを介した作用で高脂肪食摂取下の肥満とインスリン抵抗性を誘導する。

GIPは上部小腸に存在する腸管内分泌K細胞からグルコースや脂肪の摂取によって分泌されるが、特に脂肪の摂取はGIP分泌を強く誘導する(4)。また慢性的な高脂肪食肥満状態では、GIP分泌量が恒常的に増加する(5)。そこで当研究室ではGIP遺伝子欠損マウスを作製し、高脂肪食下のGIPの過分泌の抑制が肥満やインスリン抵抗性を軽減することを示した (6)。以上からGIPは「脂肪摂取」と「肥満」をつなぐ重要な消化管ホルモンであり、GIP過分泌抑制を目的とした栄養素の探索は高脂肪食肥満やインスリン抵抗性改善に有効と考えられる。

中鎖脂肪酸トリグリセリド (medium-chain fatty acid triglyceride: MCT) は、C6-12の中鎖脂肪酸 (medium-chain fatty acid [MCFA]) とグリセリンで構成されるLCTと同じエネルギー価を有する経口摂取可能な脂肪である。ヒトのメタ解析から、MCT摂取がLCT摂取と比較して体重や体脂肪量の増加を減少させることが知られている (7)。我々は、MCTの単回投与でGIPが誘導されないこと、MCTの長期摂取時にGIPの過分泌が誘導されない結果、肥満やインスリン抵抗性が誘導されないことを明らかにした (5)。しかし、GIPを産生するK細胞の詳細な解析は行われず、K細胞の特性は不明である。そこで我々はMCTの長期摂取時がK細胞に及ぼす影響について検討する

2) 戦略(Approach)

これまでin vivoでのK細胞は、肉眼で腸管上皮細胞と識別不可能であるため、K細胞の正確な評価と解析が困難であった。そこで我々は、GIP遺伝子に緑色蛍光タンパクでGFPを組み込んだK細胞可視化マウスを以前に作成し(8)、本研究に用いることで詳細なK細胞解析を可能とした。

3) 方法

・GIP分泌の評価
通常食 (10%ラードオイル)、MCT食 (40%MCTオイル)、LCT食 (40%ラードオイル)を用いてK細胞を蛍光緑色タンパクで可視化した6週齢GIP-GFP knock-inヘテロマウスに長期負荷を行う。
・体重推移
・経口ブドウ糖負荷試験(OGTT)時のGIP血中濃度
・経口コーンオイル負荷試験(OCTT)時のGIP血中濃度

1．研究概要(2)
4) 結果(Results)
負荷12週間後の体重は、高LCT食マウスで最も体重が増加し、通常食マウスに比較して40.9%の増加だった。高MCT食マウスは通常食マウスと比較して24.5%の有意な体重増加を認めたが、高LCT食マウスに比較して有意に減少した。OGTT中の血糖値は3群間で有意な差を認めなかった。GIP血中濃度は、高MCT食マウスは通常食マウスに比較して有意に高値を示したが、高LCT食マウスと有意な差を認めなかった。OCTT中のGIP血中濃度は、高LCT食マウス、通常食マウスに比較してMCT食マウスで最も高かった。OGTTおよびOCTT中のインスリンは、高LCT食マウスで高かった。以上から、高MCT食の摂取は、高LCT食の摂取に比較して体重増加は少ないが、糖や脂肪の摂取時のGIP分泌が、それぞれ同等、有意に高くなることが示された。

5) 考察(Discussion)
MCTの単回摂取ではGIPを誘導されない。一方で長期MCT負荷マウスでは、LCT食負荷マウスに比較して体重が有意に低下しているにも関わらず、糖負荷後のGIP分泌は同等であった。さらにコーンオイル負荷試験では、LCT食負荷マウスに比較して有意にMCT食負荷マウスでGIP分泌が高かった。よって長期MCT負荷マウスK細胞では栄養素に対するGIP分泌がLCT負荷マウスK細胞より高い可能性が示唆される。この原因として小腸内K細胞数増加またはK細胞での栄養素感知にかかわる分子のへ発現が増加していることが示唆される。よって今後フローサイトメトリーや免疫抗体法を用いてK細胞やK細胞内の遺伝子発現(転写因子や脂肪酸受容体、糖や脂質のトランスポーター発現など)を解析していく予定である。

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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						
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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。

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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

2019年12月の日本語能力試験（N1 レベル）に合格しました。

指導責任者（署名）　稲垣暢也　印

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

Supported by 日本財団 THE NIPPON FOUNDATION

第 41 期　研究者番号：G4109　作成日：2020 年 3 月 日

氏名	張 含鳳	Zhang Hanfeng	性別	F	生年月日 1986. 07. 27
所属機関（役職）	四川省腫瘤医院（主管護師）				
研究先（指導教官）	広島大学大学院医系科学研究科保健学分野（宮下 美香教授）				
研究テーマ	中国の生殖年齢にある男性がん患者の妊娠性温存をめざした支援プログラムの効果 Acutual situation of fertility preservation to the reproductive-aged male cancer patients in China				
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 19／22
学生本人が行った研究の概要	がん患者の妊孕性温存に関する医療者への教育プログラムについて、システマティックレビューを行い、英語論文を作成した。登録完了後に英文雑誌へ投稿する予定である。学位論文に関する研究については、男性がん患者と医療専門職者の妊孕性温存の知識、態度、行動を調べるインタビューを計画し、広島大学、勤務施設の研究倫理審査委員会の承認を得た。	
総合評価	【良かった点】 毎日、大学の研究室で博士論文作成に向けた研究活動を行っていた。プレゼンや成果物の質は高く、授業や演習にも積極的に参加した。英語だけでなく日本語での発言も増え、日本語能力は高まったと思われる。何事に対しても真面目に取り組み、指導教員への報告、連絡、相談を欠かさなかった。研究室内外の大学院生との交流を通じ、よい仲間を得られた。	
	【改善すべき点】 特にございません。	
	【今後の展望】 新型コロナの影響で予定どおり研究が進捗するか懸念されるが、現時点では計画どおり進行している。中国でのインタビュー調査の結果を踏まえ、中国での全国調査を計画し、実施した結果を博士論文として英文雑誌へ投稿する予定である。	
学位取得見込	2022 年 3 月に学位を取得する見込みである。	
評価者（指導教官名）	宮下美香 ㊞	

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41期　　研究者番号: G4109　　作成日:2020年1 月30 日

氏名	Zhang Hanfeng	張 含鳳	性別	F	生年月日 1986. 07. 27
所属機関(役職)	四川省腫瘤医院(主管護師)				
研究先(指導教官)	広島大学大学院医系科学研究科保健学分野(宮下 美香教授)				
研究テーマ	中国の生殖年齢にある男性がん患者の妊娠性温存をめざした支援プログラムの効果 Acutual situation of fertility preservation to the reproductive-aged male cancer patients in China				
専攻種別	論文博士	□	課程博士	☑	

1. 研究概要(1)

1)目的(Goal)

中国におけるがんの早期発見と治療の進歩により患者の生存率が高まり、長期的な患者のQuality of Life (以下QOLとする)を考慮した医療ケアの提供が求められている。がん治療に対する有害事象として、妊孕性への影響が挙げられる。The American Society of Clinical Oncology (ASCO)と the European Society for Medical Oncology (ESMO)は男性がん患者に対する妊孕性温存方法として、精子の凍結保存を推奨している。妊孕性を温存されたがん患者はがんへの適応がよく、妊孕性がない患者は不安、抑うつ、悲嘆を経験することが先行研究において示されている。中国では、家庭に子どもを持つことが伝統的に重要視されている。中国政府が2015年より夫婦が2人の子供を持つことを認めたことから、がん治療による妊孕性への影響に対する患者の関心は一層高まると推察される。

本研究は、中国のがん専門病院においてがん治療もしくはがん看護を行う医療専門職者、生殖医療従事者、男性がん患者における妊孕性温存に関する知識、態度、行動を明らかにすることを目的とする。本研究により、男性がん患者の妊孕性温存を促進するための基礎的な資料を得ることができる。

2)戦略(Approach)

研究担当者は半構造化面接を実施する。

3)材料と方法(Materials and methods)

<1> 研究対象者の選定方針

四川省腫瘍医院に通院中または入院中で、以下の適格基準と除外基準を全て満たす者を選定する。

A)患者

(1)適格基準

① 18歳～45歳までの男性
② 脳腫瘍、鼻咽頭がん、白血病、悪性リンパ腫、直腸がん、膀胱がん、精巣がん、前立腺がんのいずれかの診断を受けた
③ がんの病期がⅠ～Ⅲである
④ 脳腫瘍・鼻咽頭がん・白血病・悪性リンパ腫に対する化学療法もしくは放射線治療を行っている、あるいは治療終了後1年以内である。または、直腸がん・膀胱がん・精巣がん・前立腺がんに対する手術療法、化学療法、放射線療法のいずれかもしくは複数を行っている、あるいは治療終了後1年以内である。
⑤ 病名と治療の説明を受け、理解している
⑥ 中国語による会話、読み書きが行える
⑦ インタビューをICレコーダーで録音することに同意が得られる
⑧ 研究への参加に対する同意が文書で得られる

(2)除外基準

① 四川省腫瘍医院以外の病院でがんに対する治療を受けた
② 身体的健康に問題(強い痛み、嘔気など)を有するため主治医が研究参加不可能と判断する
③ 精神的健康に問題(うつ病、PTSDの既往など)を有する、もしくは精神的治療が必要と主治医もしくは精神科医により診断される
④ その他、身体・精神状態に問題があり、研究責任者または研究担当者により研究参加が不可能と判断される

B)医療専門職者

(1)適格基準

① 脳腫瘍、鼻咽頭がん、白血病、悪性リンパ腫、直腸がん、膀胱がん、精巣がん、前立腺がんのいずれかに対する治療を行う医師、がん患者への看護を行う看護師、生殖医療に従事する医師
② 1年以上がんに対する治療もしくは看護の経験がある、または1年以上生殖医療に従事した経験がある
③ インタビューをICレコーダーで録音することに同意が得られる
④ 研究への参加に対する同意が文書で得られる

<2>研究の方法

(1)研究方法

研究担当者はインタビューガイドを用いて半構造化面接を実施する。インタビューは対象者の負担を考え、30分間から1時間程度、回数は原則1回、身体・精神的に疲労を感じない程度とする。インタビューが1時間を超える場合は別日での調査を依頼するが、研究対象者の希望に応じて対応する。面接内容は対象者の承諾を得てICレコーダーに録音する。人口統計学的変数については、構造化質問紙を用いて研究担当者が聞き取る。

1．研究概要(2)
A)患者
四川省腫瘍医院の外来と入院棟(外科病棟、内科病棟、放射線科病棟)にポスターを掲示する。研究担当者が研究に関心を持った患者より電話もしくは電子メールにて連絡を受け、適格基準を満たすか確認する。適格基準を満たす場合、研究の説明を行うために会う日時の約束をいただく。四川省腫瘍医院の放射線科の個室で研究担当者が研究協力依頼の文書を用い研究の内容を説明する。研究協力への同意が得られた患者に同意文書へ署名をしてもらい、インタビューを行う日時と場所を調整する。研究担当者は対象者の希望の日時に、四川省腫瘍医院の放射線科の個室もしくは患者の入院個室でインタビューを実施する。医学的変数は診療録より情報を得る。
B)医療専門職者
① 医師、生殖医療従事者
選定基準を満たす各診療科の診療科長へ研究の協力を依頼する。診療科長に対し、研究目的および内容を文章と口頭で説明する。同意が得られた診療科の診療科長に、調査対象者の選定を依頼する。対象者に、文書と口頭で研究の趣旨、自由意思の尊重、プライバシーの厳守、結果の公表について説明し、署名による研究参加の同意を得る。研究担当者はインタビューガイドを用い、四川省腫瘍医院の放射線科の個室にて半構造化面接を実施する。
② 看護師
対象となる病棟の選定については看護部へ依頼する。承諾の得られた病棟管理者に対し、研究目的および内容を文章と口頭で説明し、調査対象者の選定を依頼する。対象者に、文書と口頭で研究の趣旨、自由意思の尊重、プライバシーの厳守、結果の公表について説明し、署名による研究参加の同意を得る。研究担当者はインタビューガイドを用い、四川省腫瘍医院の放射線科の個室にて半構造化面接を実施する。
(2)解析方法
実施した半構造化面接の録音内容を逐語録として記述し、繰り返し読み、妊孕性温存に関する知識、態度、実施状況の語りに対し、意味内容を保ち具体的な言葉でコード化する。面接の録音内容の逐語録の作成とコード化を研究担当者が行う。コード化したデータを英語に翻訳し(暗証番号でロックしたファイル)、クラウドの活用により研究責任者が受け取る。人口統計学的変数、医学的変数についても英語に翻訳し、個人が特定できないようID番号を付して暗証番号でロックしたファイルに保存し、クラウドの活用により研究責任者が受け取る。コードの意味内容の類似性に従い分類しサブカテゴリーとして命名し、さらに抽象化しカテゴリーを生成する。カテゴリー数、内容に応じ、コアカテゴリーを生成する。この際、研究責任者、研究担当者が対面もしくはWeb会議にて分析結果を吟味し、適切性を確保する。人口統計学的変数、医学的変数については、記述統計量を算出する。
(3)評価項目・方法
A)患者：妊孕性温存の知識、妊孕性温存に対する態度、妊孕性温存の情報探索行動と意思決定、医学的変数、人口統計学的変数(年齢、婚姻状況、子供の数、宗教、職業、仕事の年数、学歴、がんの病名、病期、保険の利用、家族の月収入)
B)医師、看護師：妊孕性温存の知識、妊孕性温存に対する態度、妊孕性温存の実践、人口統計学的変数(性別、年齢、仕事の年数、学歴、婚姻状況、子供の数、職名、診療科)
C) 生殖医療従事者：年間患者数、妊孕性温存の阻害因子、妊孕性温存を促進するための課題、人口統計学的変数(性別、年齢、仕事の年数、学歴、婚姻状況、子供の数、職名)
(4)研究の目標症例数
患者15例 医師8例 看護師8例 生殖医療従事者5例
設定根拠：データの飽和を得るため、先行研究から見積もった。

4)実験結果(Results)
2019年4月から9月まで、宮下先生の指導のもとで研究の計画を作成した。さらに、12月に広島大学と四川省腫瘍医院の倫理審査委員会で承認された。2020年2月から半構造化面接を実施する。

5)考察(Discussion)
なし

6)参考文献(References)
1) Griggs JJ, Sorbero ME, Mallinger JB, Quinn M, Waterman M, Brooks B et al (2007) Vitality, mental health, and satisfaction with information after breast cancer. Patient Educ Couns 66:58-66.
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3) Ajala T, Rafi J, Larsen-Disney P, Howell R (2010) Fertility preservation for cancer patients: a review. Obstet Gynecol Int 2010:160386.
4) Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, Quinn G, Wallace WH, Oktay K (2013) Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol 31:2500-2510.
5) Peccatori FA, Azim HA Jr, Orecchia R, Hoekstra HJ, Pavlidis N, Kesic Vetal (2013) Cancer, pregnancy and fertility: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 24 (Suppl 6):vi160-vi170.
6) Saito K, Suzuki K, Iwasaki A, Yumura Y, Kubota Y (2005) Sperm cryopreservation before cancer chemotherapy helps in the emotional battle against cancer. Cancer. 104:521-524.
7) Rosen A, Rodriguez-Wallberg KA, Rosenzweig L (2009) Psychosocial distress in young cancer survivors. Semin Oncol Nurs 25:268-277.
8) Lambertini M, Del Mastro L, Pescio MC, Andersen CY, Azim HA Jr, Peccatori FA et al (2016)Cancer and fertility preservation:international recommendations from an expert meeting. BMC Med 14(1):1.
9) Urech C, Ehrbar V, Boivin J, Müller M, Alder J, Zanetti Dällenbach R, Rochlitz C, Tschudin S (2018) Knowledge about and attitude towards fertility preservation in young female cancer patients: a cross-sectional online survey. Hum Fertil (Camb) 21:45-51.
10) Sallem A, Shore J, Ray-Coquard I, Ferreux L, Bourdon M, Maignien C, Patrat C, Wolf JP (2018) Fertility preservation in women with cancer: a national study about French oncologists awareness, experience, and feelings. J. Assist. Reprod. Genet 35: 1843-1850.

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さい。

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

学会名 Conference	Oncology Nursing Society (ONS) 44th Annual Congress		
演 題 Topic	Level of knowledge and needs on fertility preservation in reproductive-aged male patients with cancer		
開催日 date	2019 年 4 月 11 日	開催地 venue	Anaheim, USA
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	no		
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(署名) 宮下美香 印

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

Supported by 日本財団 THE NIPPON FOUNDATION

第 41 期　研究者番号：G4110　作成日：2020 年 3 月 日

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研究先（指導教官）	長崎大学原爆後障害医療研究所（高村 昇教授）				
研究テーマ	福島県富岡町における環境放射能モニタリングと住民の被ばく線量評価 Environmental monitoring and estimation of exposure doses of residents in Tomioka Town, Fukushima Prefecture				
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 9／35
学生本人が行った研究の概要	長崎大学が復興推進拠点を設置している福島県富岡町において、現在復興再生拠点区域に設定され、除染が進められている夜ノ森駅周辺地域における空間線量率を走行サーベイによって定期的に測定し、除染によって空間線量率が低下していることを他地域と比較しながら評価した。得られた結果は今後の福島県における復興再生拠点区域の除染、避難解除に向けた住民とのリスクコミュニケーションの際に有用な科学的根拠となると考えられる。	
総合評価	【良かった点】 研究に対して極めて真摯に向き合っており、毎日熱心に実験、論文執筆を行っている。 【改善すべき点】 特に大きな点はなし。今後さらに研究をすすめて将来的に研究指導者として成長するためには、得られたデータを正しく解析、解釈し、それを端的にまとめて提示することが極めて重要である。 【今後の展望】 今後は論文の執筆をさらに進めて学位論文を完成させ、学位取得の準備を行う予定である。	
学位取得見込	令和３年度か遅くとも令和４年度には博士（医学）の学位を取得できる予定である。	
	評価者（指導教官名） 高村 昇 ㊞	

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41期　　研究者番号: G4110　　作成日:2020年1月17日

氏名	Cui Limeng	崔 力萌	性別	F	生年月日 1986. 11. 03
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研究先(指導教官)	長崎大学原爆後障害医療研究所(高村 昇教授)				
研究テーマ	福島県富岡町における環境放射能モニタリングと住民の被ばく線量評価 Environmental monitoring and estimation of exposure doses of residents in Tomioka Town, Fukushima Prefecture				
専攻種別	論文博士	☐	課程博士	☑	

1. 研究概要(1)

1)目的(Goal)
Ascertain air dose rates and decontamination effect as well as analyses the radiocesium movement;
Determine the temporal evolution of the air dose rate in various land types;
Evaluate the effective dose for residents and workers.

2)戦略(Approach)
We carried out a detailed and high-frequency radiation monitoring program using a car-borne survey to provide relatively high-density data. We also evaluated the effects of decontamination efforts, such as reductions in ambient and radiocesium dose rates, in three areas ("Decontaminated area", "Radioactive waste storage area" and "Non-decontaminated area") with markedly different characteristics in the difficult-to-return zone in Tomioka Town.

3)材料と方法(Materials and methods)
We regularly measured the ambient dose rate from July 2018 to July 2019 (10 times in the Decontaminated area; 11 times in the Radioactive waste storage area; nine times in the Non-decontaminated area). The difficult-to-return zone of Tomioka Town was surveyed using a car-borne survey system, Radi-probe® (Model: HDS-101GN, Mirion Technologies, Inc , Japan). Combined with the output photos, the three districts were precisely divided. The measurement points ranged from 510 to 995, 747 to 1508 and 121 to 189 in the Radioactive waste storage area, Decontaminated area and Non-decontaminated area, respectively. Effective doses were determined for external exposure.

4) 実験結果(Results)
The median dose rates in the "Decontaminated area" in the difficult-to-return zone decreased rapidly from 1.0 μSv/h to 0.32 μSv/h; however, the median dose rates in the "Non-decontaminated area" and "Radioactive waste storage area" were maintained between 1.1-1.4 μSv/h and 0.46-0.61 μSv/h, respectively. The detection of cesium-137 (Cs-137) in the Decontaminated area also decreased rapidly from 64% to 6.7%. On the other hand, the detection of Cs-137 in the Contaminated area and Radioactive waste storage area decreased from 97% to 88% and 53% to 16%, respectively. We confirmed that the dose rates in the Decontaminated area dramatically decreased due to decontamination work aiming to help residents return home. Moreover, the estimated external exposure dose of workers during the present survey was 0.69 mSv/y in the Decontaminated area and 0.57 mSv/y in the Radioactive waste storage area, respectively. This case of Tomioka Town within the "difficult-to-return zone" may be the first reconstruction model for evaluating environmental contamination and radiation exposure dose rates due to artificial radionuclides derived from the nuclear disaster. The frequency distributions of the ambient dose rates within the difficult-to-return zone of Tomioka town were illustrated in Figure 1.

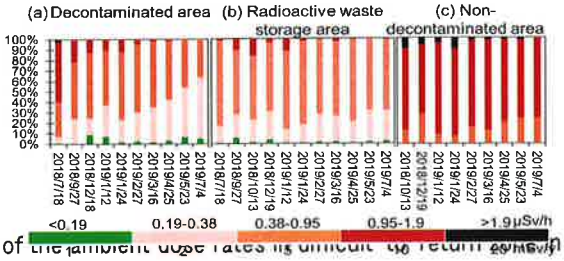

Figure 1. Relative frequencies of the ambient dose rates in difficult to return zone in Tomioka town, Fukushima prefecture during July 2018 to July 2019.

5)考察(Discussion)

The dose rates in the Decontaminated area decreased faster than those in the Radioactive waste storage area and Non-decontaminated area from July 2018 to July 2019. Significant differences in ambient dose rates were observed among surveys in the Decontaminated area, Radioactive waste storage area and Non-decontaminated area ($p<0.001$). Noticeable fluctuations in dose rates in the Radioactive waste storage area and Non-decontaminated area were observed. Also, a relatively stable downward trend was observed in the Decontaminated area.

The main reason for the decrease in dose rates over this 1-year period in Yonomori District is the decontamination efforts which have focused on removing deposits from roofs, decks and gutters; wiping off roofs and walls; high-pressure washing of houses and buildings; mowing lawns; removing fallen leaves and stripping topsoil in gardens; removing deposits in ditches and high-pressure washing of roads. In the present study, the estimated annual effective dose of decontamination workers, as well as the residents of decontaminated areas, was lower than the annual effective dose limits recommended by the Japanese government. Nevertheless, radiation safety education for workers is needed to appropriately protect them from radiation.

6)参考文献(References)

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2.Ministry of the Environment of Japan. Additional exposure doses after an accident (example of calculation).(Accessed August 23, 2019). https://www.env.go.jp/en/chemi/rhm/basic-info/1st/02-04-09.html (Accessed July 31, 2019) (2018).

3.Profes, E. et al. Nuclear power facilities such as disaster prevention measures consignment expenses (measurement of continuous air dose rate that simulated life action pattern) (in Japanese). (2017) 4.Ministry of the Environment of Japan. Shielding and reduction coefficient. (Accessed August 23, 2019). https://www.env.go.jp/en/chemi/rhm/basic-info/1st/02-04-08.html (Accessed July 31, 2019) (2018). 5.Ministry of health, labour and welfare, Japan. Guidelines on Prevention of Radiation Hazards for Workers Engaged in Decontamination Works.https://www.mhlw.go.jp/english/topics/2011eq/workers/ri/gn/gn_141118_a01.pdf (Accessed August 23, 2019).

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	Chemical content and source apportionment of 36 heavy metal analysis and health risk assessment in aerosol of Beijing					
掲載誌名 Published journal	Environmental Science and Pollution Research					
	2019 年 12 月	巻(号)	頁 ～ 頁		言語 Language	English
第1著者名 First author	Limeng Cui	第2著者名 Second author	Zhuona Wu	第3著者名 Third author	Peng Han	
その他著者名 Other authors	Yasuyuki Taira, Huan Wang, Qinghua Meng, Zechen Feng, Shuguang Zhai, Jun Yu, Weijie Zhu, Yuxia Kong, Hongfang Wang, Hong Zhang, Bin Bai, Yun Lou, Yongzhong Ma					
論文名 2 Title	日本福岛第一核电站事故七年后环境放射性水平与公众健康情况的现状及启示 Situation and enlightenment in an environmental radioactivity and public health perspective seven years after Fukushima nuclear power plant accident					
掲載誌名 Published journal	中华放射医学与防护 Chinese Journal of Radiological Medicine and Protection					
	2019 年 8 月	39(8) 巻(号)	619 頁 ～ 623 頁		言語 Language	Chinese
第1著者名 First author	崔力萌	第2著者名 Second author	高村升	第3著者名 Third author	马永忠	
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 meeting

学会名 Conference	日本放射線影響学会第62回大会				
演 題 Topic	Environmental Remediation of a Restricted Area in Tomioka Town, Fukushima Prefecture				
開催日 date	2019 年 11 月 16 日	開催地 venue	京都大学		
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（署名） 高村 岳 ㊞

Chemical content and source apportionment of 36 heavy metal analysis and health risk assessment in aerosol of Beijing

Limeng Cui, Zhuona Wu, Peng Han, Yasuyuki Taira, Huan Wang, Qinghua Meng, Zechen Feng, Shuguang Zhai, Jun Yu, Weijie Zhu, Yuxia Kong, et al

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RESEARCH ARTICLE

Chemical content and source apportionment of 36 heavy metal analysis and health risk assessment in aerosol of Beijing

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Received: 15 February 2019 / Accepted: 4 September 2019

Abstract

The concentration levels of 36 airborne heavy metals and atmospheric radioactivity in total suspended particulate (TSP) samples were measured to investigate the chemical characteristics, potential sources of aerosols, and health risk in Beijing, China, from September 2016 to September 2017. The TSP concentrations varied from 6.93 to 469.18 μg/m^3, with a median of 133.97 μg/m^3. The order for the mean concentrations of heavy metals, known as hazardous air pollutants (HAPs), was as follows: Mn > Pb > As > Cr > Ni > Se > Cd > Co > Sb > Hg > Be; Non-Designated HAPs Metals: Ca > Fe > Mg > Al > K > Na > Zn > P > Ba > Ti > Cu > Sr > B > Sn > I > V > Rb > Ce > Mo > Cs > Th > Ag > U > Pt. The median concentration of As was higher than China air quality standard (6 ng/m^3). The gross α and β concentration levels in aerosols were (1.84 ± 1.59) mBg/m^3 and (1.15 ± 0.85) mBg/m^3, respectively. The enrichment factor values of Cu, Ba, B, Ce, Tl, Cs, Pb, As, Cd, Sb, Hg, Fe, Zn, Sn, I, Mo, and Ag were higher than 10, which indicated enriched results from anthropogenic sources. Pb, As, and Cd are considered to originate from multiple sources; fireworks released Ba during China spring festival; Fe, Ce, and Cs may come from stable emissions such as industrial gases. The health risks from anthropogenic metals via inhalation, ingestion, and dermal pathway were estimated on the basis of

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health quotient as well as the results indicated that children faced the higher risk than adults during the research period. For adults, the health risk posed by heavy metals in atmospheric particles was below the acceptable level.

Keywords Heavy metals, · Atmospheric radioactivity, · Enrichment factor, · Hazard quotient

Introduction

Air pollution is a global threat with negative effects on public health and ecosystems (European Environment Agency 2018). Severe air pollution not only increases the risk of cancer, but also may lead to cardiovascular or chronic obstructive pulmonary disease, allergies, and Alzheimer's disease (WHO 2013a; Sun et al. 2014b; Morishita et al. 2015; Zhang et al. 2016; Kilian and Kitazawa 2018). Previous studies suggested the cardiovascular effects of ambient air particulate matter (PM) are greatly influenced by their metal contents (nickel) (Zanobetti et al. 2009; Mostofsky et al. 2012).

Some heavy metals in atmospheric particles can severely affect human health (WHO 2000, 2013b; U.S. EPA 2016, 2019a). For instance, arsenic (As) can increase incidence of lung cancer (WHO 2000); lead (Pb) can adversely affect the nervous system, kidney function, immune system, reproductive and developmental systems, and cardiovascular system (UNEP, 2010; U.S. EPA 2007); mercury (Hg) intake in China leads to fetus intelligence quotient decrements and fatal heart attacks (Chen et al. 2019). Although some metallic elements, such as iron (Fe), are indispensable to the human body, excessive amounts of these metals still present health risks (Geiger and Cooper 2010).

Atmospheric radioactivity originated from the naturally occurring radioisotopes, such as Thorium-232 (^{232}Th) and Uranium-238 (^{238}U) series and their decay products, nuclear accident, and nuclear testing (UNSCEAR 2000; Tzortzis and Tsertos 2004). The inhalation of radioactive atmospheric particles is one of the natural radiation sources for human beings (UNSCEAR 2000). Therefore, gross alpha (α) and beta (β) are generally measured for screening unusual radioactivity in the atmosphere (Dueñas et al. 1999).

Beijing, as the capital of China, has a high population density and the largest vehicle ownership rates in China (about 6 million and 80 thousand vehicles in 2018) (The People's Government of Beijing Municipality 2018; Beijing Traffic Management Bureau 2019). From 2013 to 2017, the fine particle pollution decreased from 89.5 to 58 μg/m^3 in Beijing but still exceeds the national standard by 66% (Fig. 1) (UN environment 2019). Furthermore, the provinces around Beijing, such as Tianjin and Hebei, have a relatively large industrial emission (Li et al. 2018a; Yang et al. 2019). Previous studies in Beijing reported high health risks and related health impact caused by heavy metals in air particulate matter (Langrish et al. 2009; Rich et al. 2012; Du et al. 2013; Shao et al. 2017; Li et al. 2018b; Yue et al. 2019). Therefore, the study of heavy metals in atmospheric particles is significant to the haze pollution control and human health protection.

Thus, in this study, we analyzed the levels of metals and gross radioactivity of total suspended particulate (airborne particles with diameters less than 100 μm, TSP) samples in Beijing, China. Moreover, possible risk sources were identified and analyzed. Finally, the results of element concentrations were also used to develop a quantitative estimate of the health quotients (HQs).

Methodology

Air sampling collection and metal measurement

The measurements occurred on the rooftop of an office building (20 m above ground) at Hepingli Zhongjie, Dongcheng District, Beijing (116.2° E, 39.6° N) (Fig. 1). The site is located in a mixed-use neighborhood including schools, residences, and parks. The site is also in close proximity to two major streets, i.e., the second ring road around Beijing (approximately 1 km south) and the third ring road (approximately 2 km north).

Seventy-five TSP samples were collected by a high-volume air sampler (Senya, Sweden, Snow White, 900 m^3/h) from September 2016 to September 2017. The collecting time for each sample was 24 h. To analyze the seasonal variation, the average of each season (spring: n = 15, 2017/3/2–2017/5/18; summer: n = 11, 2017/6/3–2017/8/25; autumn: n = 26,2016/9/28–2016/11/29, 2017/9/3–2017/9/22; winter: n = 21, 2016/12/2–2017/2/23) was used to draw a percentage stacked column chart (Fig. 1). The air volume that passed through the sampler (10-μm pore size) was 13709~26090 m^3/day.

In this work, 36 metal elements were analyzed: manganese (Mn), arsenic (As), cadmium (Cd), nickel (Ni), chromium (Cr), lead (Pb), selenium (Se), antimony (Sb), mercury (Hg), beryllium (Be), cobalt (Co), iron (Fe), calcium (Ca), magnesium (Mg), aluminum (Al), potassium (K), sodium (Na), zinc (Zn), phosphorus (P), barium (Ba), titanium (Ti), copper (Cu), strontium (Sr), boron (B), tin (Sn), iodine (I), vanadium (V), rubidium (Rb), cerium (Ce), molybdenum (Mo), thallium (Tl), cesium (Cs), thorium (Th), argentum (Ag), uranium (U), platinum (Pt). The results of heavy metals were divided to hazardous air pollutant (HAP) group and non-designated HAP group on the basis of *the Initial List of Hazardous Air Pollutants with Modifications* (U.S.EPA 2010; U.S. EPA 2016). Mn, Pb, As, Cr, Ni, Se, Cd, Co, Sb, Hg, and Be and

Fig. 1 The map of Beijing and the surroundings with sampling point (red triangle). L. M. C. created the map using the software Green Map® (Tokyo Shoseki Co., Ltd., Tokyo, Japan)

their compounds are included in the list. Although the other 25 metals have not been designated as hazard air pollution yet, excessive amounts of these metals still present health risks.

The net weight of TSP was obtained by weighing the filter membrane after sampling and subtracting the membrane weight before sampling. The TSP concentrations were obtained by dividing the net sampling weight by the sampling flow rates.

Air pollution data (PM2.5, PM10) were obtained from the website of Beijing municipal ecological environmental bureau (Beijing Municipal Ecological Environmental Bureau 2019). The temperature and relative humidity during sampling were derived from the website of Wunderground website (www.wunderground.com).

A low background alpha, beta measurement apparatus is a lower cost device that is widely used in environmental sample monitoring. By using standard sampling methods and standard ways of processing and storing, we choose Americium-241 (Am-241) and Potassium-40 (K-40) as standard materials to conduct the experiment by employing qualified drugs and reagents. To ensure the veracity of the method, all devices and instruments that are involved are calibrated by the National Institute of Metrology and are still in the validity period.

The elemental analysis was performed using 7700x Agilent inductively coupled plasma mass spectrometry (ICP-MS, American) and Mass Hunter Workstation Software (Version: A.01.02; Agilent Technologies). Calibrants were prepared from multi-element standard solution (Lot: S130823001,Canada, Plasma CAL). The samples (including blank membrane samples) were digested by adding 10 mL concentrated nitric acid and digested according to the microwave procedure. After the acid wiped out, volume was 50 mL with pure water for determination. Quantitative analysis of the elemental concentrations in unknown samples was measured by an internal standard method.

Enrichment factor and health risk assessment

To determine whether the presence of a certain element was due to natural or anthropogenic sources, the enrichment factor (Ef) value was eliminated to indicate the source identification of heavy metal abundances in the atmosphere. Al is used as a reference element since it is ubiquitous in the environment and has no significant anthropogenic sources. The Ef of heavy metals can be calculated using the following equation (Taylor S.R 1964; Hsu et al. 2010):

$$\mathrm{Ef} = \frac{(\mathrm{C/Al})_{\mathrm{aerosol}}}{(\mathrm{C/Al})_{\mathrm{Crust}}} \quad (1)$$

where $(C/Al)_{aerosol}$ is the concentration ratio of given heavy metals C to Al in ambient samples, and $(C/Al)_{crust}$ is the same ratio of the heavy metal C to Al in the average samples. The background concentrations of heavy metals in the background are selected in China (Li Tong 1997).

Previous studies show that Ef values lower than 10 suggest a greater possibility of pollution from natural crustal elements, while values between 10 and 100 should be considered to indicate that elements are from human activities and mixed sources (from both natural and anthropogenic sources); high Ef values (> 100) are considered to be the result of anthropogenic sources or exceptional geological events (Betha et al. 2014; Lyu et al. 2017). However, studies support different standards with Ef values between 2 and 10 being suggestive of moderate mixed sources (Li Tong 1997; Lin et al. 2016).

Human health can be significantly influenced by heavy metals in the atmosphere via ingestion, dermal contact, and inhalation (WHO 2000). The exposure parameters for exposure assessment models are referenced from the U.S. EPA, environmental site assessment guidelines, and other relative studies (U.S. EPA 2009; Du et al. 2013; Sun et al. 2014a; Wei

et al. 2015; Zheng et al. 2015; Zhang et al. 2016; Megido et al. 2017; Kicinska and Bozecki 2018).

The average daily dose (mg/kg day^{-1}, ADD) was estimated for each element using the following expressions:

$$\mathrm{ADD_{ing}} = \frac{\mathrm{C} \times \mathrm{IR_{ing}}}{\mathrm{BW}} \times \frac{\mathrm{EF} \times \mathrm{ED}}{\mathrm{AT}} \times \mathrm{CF} \quad (2)$$

$$\mathrm{ADD_{derm}} = \frac{\mathrm{C} \times \mathrm{SA} \times \mathrm{AF} \times \mathrm{ABS}}{\mathrm{BW}} \times \frac{\mathrm{EF} \times \mathrm{ED}}{\mathrm{AT}} \times \mathrm{CF} \quad (3)$$

$$\mathrm{ADD_{inh}} = \mathrm{C} \times \mathrm{IR_{inh}} \times \frac{\mathrm{EF} \times \mathrm{ED}}{\mathrm{BW} \times \mathrm{AT_n}} \times \mathbf{CF} \quad (4)$$

where C (ng/m^3) is the metal concentration in TSP; IR is the ingestion rate (100 mg/day for adults and 200 mg/day for children), and inhalation rate (20 m^3/day for adults and 5 m^3/day for children) (Vik et al. 1999; Du et al. 2013); BW is the average body weight of Beijing citizen (66.1 kg for adults and 22.7 kg for children) (He et al. 2016; Meng Jie et al. 2017); EF is the exposure frequency (350 days/year) (U.S. EPA 2014); ED is the exposure duration (24 years for adults and 6 years for children) (U.S. EPA 2014); AT is the average time (365 days × ED); CF is the conversion factor (1×10^{-6} kg/mg) (U.S. EPA 1989); SA is the surface exposure area of Chinese in summer (4020 cm^2 for adults and 2160 cm^2 for children) (Zong et al. 2009); AF is the adherence factor (0.07 mg/cm^2/day for adults and 0.02 mg/cm^2/day for children) (U.S. EPA 2004); ABS is the dermal absorption factors (0.03 (As), 0.001 (Cd), 0.01 (others)) (Hu et al. 2012; Megido et al. 2017; U.S. EPA 2019b); AT_n = ED × 365 days× 24 h/day.

The assessment of potential health risks uses the following equation (U.S. EPA 2009):

$$\mathrm{HQ_{sum}} = \sum_{i=1}^{3} \mathrm{HQ}_i = \sum_{i=1}^{3} \frac{\mathrm{ADD}_i}{\mathrm{RfD}_i} \quad (5)$$

where RfD refers to the reference dose for the pathways which are listed in Table 1. HQ ≤ 1 indicates no adverse health effects, and HQ ≥ 1 shows a probability of adverse health effects (U.S. EPA 2001).

In this study, the hazard quotient was calculated only for heavy metals with Ef values greater than 10, which are from anthropogenic sources. Although the Fe, Mg, Ca, K, and Na are essential human nutrients and are toxic only at very high doses, we calculate the HQ for Fe due to the higher Ef value (U.S. EPA 1989).

Statistical analysis

Mean, standard deviation, and minimum and maximum values of air pollutant concentrations were calculated for descriptive statistics. Spearman's non-parametric rank order correlation coefficient was used to describe the correlation among TSP, temperature, humility, seasonal variations, gross α and β, and heavy metals. The regression lines were used to calculate the percentage of PM_{10} and $PM_{2.5}$ in TSP. The criterion for statistical significance was $p < 0.05$. Statistical analysis was performed using a SPSS 25 (IBM Corp., Armonk, NY, USA).

Results and discussion

The concentration of heavy metals and gross radioactivity

The TSP, gross radioactivity, and concentration of metal elements in the aerosol samples collected in Beijing from September 2016 to September 2017 are reported in Table 2.

During the sampling period, the TSP concentrations varied from 6.93 to 469.18 μg/m^3, with a median of 133.97 μg/m^3. The $PM_{2.5}$ and PM_{10} concentrations obtained from the website of Beijing municipal ecological environmental bureau ranged from 6 to 430 μg/m^3 (6–510 g/m^3), with a median of 52 μg/m^3 (79 μg/m^3) (Beijing Municipal Ecological Environmental Bureau 2019). The $PM_{2.5}$ and PM_{10} concentrations were compared with the TSP concentrations to determine the proportion in TSP from the regression lines. The results show that, in this study, the $PM_{2.5}$ and PM_{10} took about 48% and 68% of TSP samples, respectively.

The average concentration of radioactivity in this research (gross α, 1.84 mBg/m^3; gross β, 1.15 mBg/m^3) was still clearly higher in the majority. Previous studies conducted in Qinshan nuclear power plant, Spain, and New Mexico have reported that the average of gross α and gross β ranged from 0.069 to 0.357 mBg/m^3 and 0.45 to 1.0 mBg/m^3, respectively (García-Talavera et al. 2001; Hernández et al. 2005; Bin et al. 2007; Huang et al. 2009; Thakur and Mulholland 2011). It should be noticed the average concentrations of natural radon in modern buildings is about 50 Bq/m^3 which is extremely higher than the gross α and β concentration in outdoor air (Malinovsky et al. 2018). The health risks caused by the inhalation of radioactive particles in the air are mainly considered indoor source rather than outdoor.

For Pb, Cd, and Hg, although the median values did not exceed limits of China (Pb 0.5 μg/m^3, Cd 5 ng/m^3, Hg 50 ng/m^3) (Ministry of Environmental Protection (China) 2012), the mean concentration of Pb exceeded the ambient air quality standard of the USA (0.15 μg/m^3) (U.S. EPA 2019). Compared with the WHO proposed limit values (0.5 μg/m^3 for Pb, 1 μg/m^3 for Mn, 4–13 ng/m^3 for As, 5 ng/m^3 for Cd, 1 μg/m^3 for Hg, and 20 ng/m^3 for Ni) and China air quality standard (6 ng/m^3 for As), the median concentrations of Pb, Mn, Cd, Hg, and Ni were lower than the limit except As (Van Leeuwen 2002; Ministry of Environmental Protection (China) 2012; WHO 2013a, 2017; Padoan et al. 2016). The average concentrations of As and Pb were higher than other studies

Table 1 Reference factors for assessing hazard quotient (U.S. EPA 2009, 2011, 2019b; Du et al. 2013; Sun et al. 2014a; Wei et al. 2015; Zheng et al. 2015; Zhang et al. 2016; Megido et al. 2017; Liu et al. 2018; Kicinska and Bozecki 2018)

Element	RfD_{der}—dermal reference	RfD_o—oral reference	RfC_i—inhalation reference
Pb	5.2E−04	3.5E−03	
As		3.00E−04	1.50E−05
Cd	1.00E−05	1.00E−05	1.00E−03
Sb		4.00E−04	3.0E−04
Hg		1.60E−04	3.0E−04
Fe		7.00E−01	
Zn	6E−02	3.00E−01	
Ba		2.00E−01	5.0E−04
Cu	1.2E−02	4.00E−02	
B		2.00E−01	2.0E−02
Sn		6.00E−01	
I		5.04E−03	
Mo		5.00E−03	4.0E−04
Tl			1.0E−04
Ag		5.00E−03	

conducted in Beijing during 2016 and 2017 which was reverse for Cr and Fe (Liu et al. 2018; Men et al. 2018; Jin et al. 2019). Although those researches were conducted in Beijing during a similar time period, the results showed difference which the heavy metals were considered to regional differences of pollution sources.

Seasonal distribution pattern and source analysis

The percentage of gross α and β, Pb, As, Se, Cd, Sb, Hg, K, Na, Ba, Cu, Sr, B, I, Mo, and Tl in winter exceed 50% of the whole year (Fig. 2). Gross α, gross β, Pb, As, Cr, Se, Cd, Sb, Hg, K, Na, Zn, Ba, Cu, Sr, B, Sn, I, Mo, Tl, Ag, and Pt are significantly correlated with seasonal variations ($p < 0.05$).

There were negative associations between temperature and concentration of heavy metals except Sn as well as between temperature and gross radioactivity ($p < 0.05$). The concentrations of Pb, As, Se, Cd, Sb, Hg, Zn, Sn, Mo, Tl, and Ag were correlated with humidity ($p < 0.05$). The statistical results of the Spearman correlation between gross radioactivity and 36 metal elements showed positively correlations ($p < 0.05$).

Figure 3 shows the enrichment factor (Ef) of each element calculated to evaluate the anthropogenic influence. On the basis of the mean concentration of elements, the Ef values of Cu, Ba, B, Ce, Tl, and Cs were between 10 and 100, which indicated anthropogenic sources instead of crustal sources. The Ef values of Pb, As, Cd, Sb, Hg, Fe, Zn, Sn, I, Mo, and Ag were higher than 100, which indicated highly enriched results from anthropogenic sources.

In China, metal elements in coal include I, Be, Cr, Co, Ni, Cu, As, Se, Sr, Mo, Cd, Sb, Cs, Hg, Pb, Th, U, and Ba (Dai et al. 2012; Gao et al. 2018). Some research suggested the use of Cr, Ni, Hg, and As as markers of coal combustion in China (Tian et al. 2010; Kittner et al. 2018), and coal combustion emissions are considered the main source of pollution in Beijing (Cai et al. 2017). Cd, Cu, Pb, Zn, As, and Ni were suggested to be associated with diesel and gasoline exhaust fumes from local traffic and other anthropogenic emissions (Valavanidis et al. 2006; Men et al. 2018). A study suggests that anthropogenic sources such as brake wear, tire dust, road abrasion, and fossil fuel combustion spread Cu, Sb, Pb, and Zn (Dehghani et al. 2017). Tl is considered a characteristic element of heavy industries (Lin et al. 2016). Cu, Sn, and Ag were usually used as solder alloys (Miller et al. 1994).

In winter, 21 (As, Cd, Fe, Pb, Zn, Hg, Sb, Cu, Mo, Ag, Sn, I, Cs, Ce, Tl, B, Ba, Pt, P, and Ca) of the 36 trace metals are predominantly of anthropogenic origin, with concentrations dependent on the level of anthropogenic activities. Ba, Pt, P, and Ca (Ef: 24.5, 10.5, 10.5, 10.9, respectively) showed anthropogenic origin in winter only. Pt is the major constituent of automotive catalysts (Nischkauer et al. 2017). If this is the reason for the increase of Ef value, it should be reflected in four seasons; therefore, these three elements are considered to be more easily affected by air diffusion. It should be noted that the highest concentration of Ba (the Ef values are lower than 10 in other seasons) was in February 1, 2017, which was the day of the Chinese Spring Festival when people used fireworks containing Ba to celebrate. The Ef values of I, Hg, Mo, Sb, and Zn are higher than 10, have an obvious correlation with seasons, and belong to coal metal elements (Tian et al. 2010; Dai et al. 2012; Gao et al. 2018; Kittner et al. 2018). Therefore, according to these elements, coal combustion emissions are considered to be the main source.

Most elements with an Ef value greater than 10 are significantly correlated with seasonal variations exempting Fe, Ce, and Cs. This suggests these three elements may come from stable emissions such as industrial gases. This is consistent with the findings of Yu-Chi Lin et al., which suggested the source of Fe in Beijing was mainly from iron and steel manufacturing (Lin et al. 2016). Ce is widely used as an automobile exhaust purification catalyst (Jung et al. 2005). At

Table 2 Mean, minimum, and maximum concentrations of each element determined in PM_{10} samples in Beijing from September 2016 to September 2017. All values, except for TSP, and gross α and β, are expressed in ng/m^3. TSP is expressed in μg/m^3. Gross α and β are expressed in mBg/m^3

		Median	Min	Max	Mean	SD
TSP		1.34E+02	6.93E+00	4.69E+02	1.52E+02	1.04E+02
Gross α		1.62E+00	1.45E−02	9.41E+00	1.84E+00	1.59E+00
Gross β		9.97E−01	1.33E−02	3.91E+00	1.15E+00	8.54E−01
Hazardous air pollutants (HAPs)	Mn	1.64E+02	2.51E+01	2.17E+03	2.12E+02	2.6E+02
	Pb	9.79E+01	2.87E+00	3.22E+03	1.93E+02	3.9E+02
	As	1.05E+01	4.55E−01	3.88E+02	2.70E+01	5.1E+01
	Cr	1.65E+01	1.94E+00	2.50E+02	2.22E+01	3.0E+01
	Ni	9.12E+00	1.08E+00	1.40E+02	1.20E+01	1.7E+01
	Se	5.00E+00	1.12E−01	1.71E+02	1.00E+01	2.1E+01
	Cd	2.11E+00	4.73E−02	8.79E+01	4.70E+00	1.1E+01
	Co	2.46E+00	2.72E−01	4.08E+01	3.50E+00	4.8E+00
	Sb	9.95E−01	9.06E−02	9.04E+01	3.30E+00	1.1E+01
	Hg	2.32E−01	7.51E−03	1.11E+01	5.00E−01	1.3E+00
	Be	1.60E−01	1.95E−02	2.83E+00	2.00E−01	3.0E−01
Non-designated HAPs Metals	Ca	1.22E+04	1.81E+03	1.23E+05	1.47E+04	1.5E+04
	Fe	4.77E+03	6.25E+02	5.64E+04	6.17E+03	7.0E+03
	Mg	2.66E+03	4.01E+02	2.72E+04	3.41E+03	3.4E+03
	Al	2.06E+03	3.12E+02	2.42E+04	2.97E+03	3.2E+03
	K	1.65E+03	1.25E+02	3.29E+04	2.77E+03	4.4E+03
	Na	1.16E+03	6.19E+01	5.42E+04	2.52E+03	6.4E+03
	Zn	3.04E+02	1.32E+01	6.80E+03	4.76E+02	8.1E+02
	P	1.88E+02	2.32E+01	3.16E+03	2.53E+02	3.7E+02
	Ba	1.18E+02	1.43E+01	2.23E+03	2.06E+02	3.5E+02
	Ti	9.54E+01	1.34E+01	1.29E+03	1.24E+02	1.6E+02
	Cu	6.79E+01	7.62E+00	1.56E+03	1.15E+02	1.9E+02
	Sr	4.04E+01	4.48E+00	7.27E+02	6.47E+01	1.0E+02
	B	1.17E+01	6.30E−01	7.18E+02	3.05E+01	8.4E+01
	Sn	1.04E+01	1.69E+00	2.07E+02	1.68E+01	2.5E+01
	I	5.72E+00	2.46E−01	1.97E+02	1.15E+01	2.4E+01
	V	9.26E+00	1.02E+00	8.44E+01	1.11E+01	1.1E+01
	Rb	6.70E+00	6.68E−01	9.41E+01	9.20E+00	1.2E+01
	Ce	5.57E+00	7.86E−01	1.12E+02	8.50E+00	1.3E+01
	Mo	3.32E+00	2.70E−01	6.73E+01	5.20E+00	8.3E+00
	Tl	1.04E+00	2.98E−02	3.88E+01	2.10E+00	4.7E+00
	Cs	8.44E−01	8.57E−02	1.19E+01	1.20E+00	1.6E+00
	Th	7.89E−01	1.04E−01	1.04E+01	1.11E+00	1.32E+00
	Ag	3.44E−01	1.13E−02	7.67E+00	6.00E−01	1.0E+00
	U	2.46E−01	2.73E−02	4.07E+00	3.44E−01	4.95E−01
	Pt	1.85E−03	9.00E−06	9.77E−03	2.20E−03	1.9E−03

present, the largest use of non-radioactive Cs is as a specialty high-density component in drilling mud used for petroleum exploration (Butterman et al. 2004). The industrial enterprises were the main reason for concentrations of Fe, Ce, and Cs in Beijing.

The higher contents of elements in aerosols are considered to originate from multiple sources. Firstly, in Beijing, the sources of Cd may come from burning fossil fuels, municipal waste material incineration, tire wear friction, and cigarette smoking (Geiger and Cooper 2010; Men et al. 2018). Secondly, Pb and As were anthropogenic in origin and changed with seasons. Potential sources include coal, motor vehicles, and industrial operations (Valavanidis et al. 2006; Dehghani et al. 2017). Furthermore, the industrial As include wood preserving industry paints, dyes, metals, drugs, soaps, and semi-conductors (Geiger and Cooper 2010). Although leaded gasoline has been banned in some megacities in China, Pei-Hsuan Yao et al. suggests that local unleaded fuel combustion still was a Pb

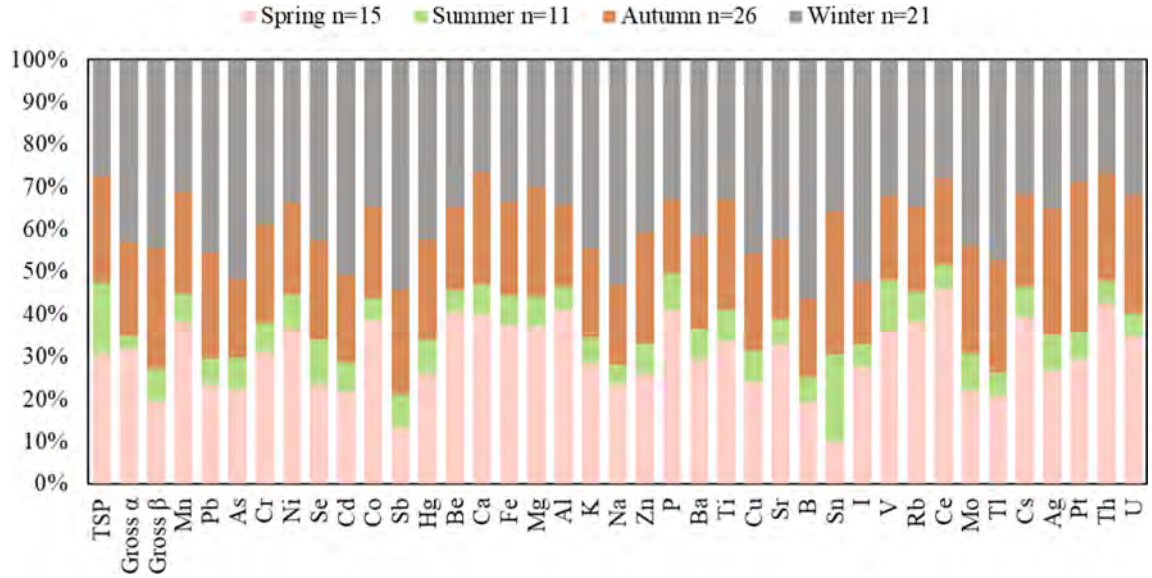

Fig. 2 The distribution of average concentration of gross radioactivity and metal elements in different seasons

contributor to the metropolitan air (Yao et al. 2015). Ore and metal processing as well as piston-engine aircraft operations using leaded aviation fuel also are considered lead sources (U.S. EPA). The seasonal differences of Pb in this study indicates that the main source of Pb in Beijing may not only come from stable release but the atmospheric dispersion conditions and coal combustion emissions in winter (Geiger and Cooper 2010).

Hazard quotient

Table 3 showed the hazard quotients of anthropogenic source metals (Pb, As, Cd, Sb, Hg, Fe, Zn, Ba, Cu, B, Sn, I, Mo, Tl, and Ag) via ingestion, dermal contact, and inhalation for children and adults. The mean HQ of As was the highest among both children and adults. The order of HQ in children and adult groups is As, Pb, Ba, Fe, Sb, Cu, Hg, I, Zn, Mo, Cd, Tl, B, Ag, and Sn and As, Ba, Pb, Sb, Fe, Cd, Tl, Cu, Mo, Hg, I, Zn, B, Ag, and Sn.

For adults, the average values of HQ for none of the metals exceeded 1, indicating the health risk posed by heavy metals in atmospheric particles was acceptable during the research period. However, the integrated risks of these metals were higher to children (1.98), while the risks through ingestion were 1.48. The contribution of risks through ingestion to HQ were 74.7% and 25.6% for children and adults, respectively. The higher ingestion rate of children was supposed to be the main reason and similar results were also obtained by other scholars (Lyu et al. 2017; Men et al. 2018). The results confirmed that from September 2016 to September 2017, the air pollution problems in Beijing was still serious for children.

Uncertainty and limitations

The risk estimation in this study has certain limitations and may have a degree of uncertainty, because only one sample site in the city center was used and all applied parameters are assumed to be ideal. It will more reasonable if sampling sites were separated in different functional areas such as industrial

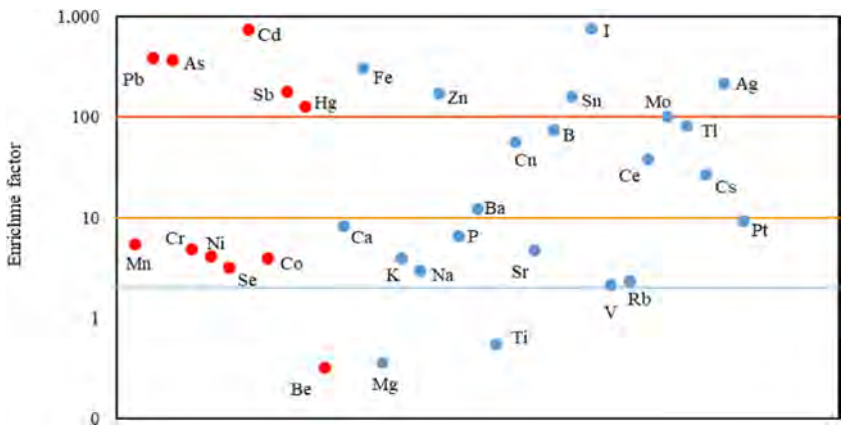

Fig. 3 Average enrichment factor values for HAP metals (red points) and non-designated HAP metals (blue points) collected in Beijing from September 2016 to September 2017

Table 3 The average hazard quotient (HQ) from heavy metals in TSP via inhalation (inh), ingestion (ing), and dermal contact (derm)

		HQ_{inh}		HQ_{ing}		HQ_{derm}		HQ_{sum}	
		Adults	Children	Adults	Children	Adults	Children	Adults	Children
	Pb	1.6E−02	1.2E−02	8.0E−02	4.7E−01	1.5E−02	6.7E−03	1.1E−01	4.8E−01
Hazardous Air Pollutants (HAP)	As	5.2E−01	3.8E−01	1.3E−01	7.6E−01	6.3E−03	2.8E−03	6.6E−01	*1.1E+00*
	Cd	1.4E−06	1.0E−06	6.8E−04	4.0E−03	7.7E−03	3.4E−03	8.4E−03	7.4E−03
	Sb	3.2E−03	2.3E−03	1.2E−02	6.9E−02			1.5E−02	7.2E−02
	Hg	4.5E−04	3.3E−04	4.2E−03	2.5E−02			4.7E−03	2.5E−02
Non−designated HAP metals	Fe			1.3E−02	7.4E−02			1.3E−02	7.4E−02
	Zn	4.7E−04	3.4E−04	2.3E−03	1.4E−02	3.3E−04	1.5E−04	3.1E−03	1.4E−02
	Ba	1.2E−01	8.7E−02	1.5E−03	8.7E−03			1.2E−01	9.6E−02
	Cu	8.3E−04	6.1E−04	4.2E−03	2.4E−02	3.9E−04	1.7E−04	5.4E−03	2.5E−02
	B	4.4E−04	3.2E−04	2.2E−04	1.3E−03			6.6E−04	1.6E−03
	Sn			4.1E−05	2.4E−04			4.1E−05	2.4E−04
	I			3.3E−03	1.9E−02			3.3E−03	1.9E−02
	Mo	3.8E−03	2.8E−03	1.5E−03	8.9E−03			5.3E−03	1.2E−02
	Tl	6.2E−03	4.5E−03					6.2E−03	4.5E−03
	Ag			1.8E−04	1.0E−03			1.8E−04	1.0E−03
SUM		6.73E−01	4.90E−01	2.54E−01	*1.48E+00*	2.98E−02	1.33E−02	9.56E−01	*1.98E+00*

The italicized values means a probability of adverse health effects

zone and a residential zone. In addition, the differences (TSP vs $PM_{2.5}$) exist in particle bound elements because of the pore size of filter (10 μm) in this study. Future studies need to consider the health risks posed by airborne heavy metals in $PM_{2.5}$. Because the measurement station is 20 m above the ground, the risk estimates may be biased compared to the risks of real public outdoor activities.

Despite these shortcomings, the risk model and conclusions of this study provide a basis for assessing and future monitoring of human health risk associated with metal exposure in Beijing, China.

Conclusions

In this study, 36 elements were measured, including some previously neglected elements, such as Ce, Cs, I, and Ag, to provide more comprehensive data to examine air pollution sources. In addition, the health risks caused by the inhalation are mainly considered indoor source rather than outdoor. Furthermore, on the basis of the enrichment factors, we confirmed that Pb, As, Cd, Sb, Hg, Fe, Zn, Cu, Ba, B, Sn, I, Mo, Ce, Tl, Cs, and Ag were the anthropogenic heavy metal aerosols. The health risks posed by heavy metals in atmospheric particles were below the acceptable level for adults as well as the children faced higher health risk than adults. Further research is needed because there is concern about health effects due to air pollution.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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· 放射卫生 ·

日本福岛第一核电站事故七年后环境放射性水平与公众健康情况的现状及启示

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【摘要】 2011 年 3 月 11 日,地震和海啸袭击日本东北部,造成福岛第一核电站发生大量放射性物质释放到环境中的严重事故。本文对后福岛时期的环境监测方式、环境数据(环境 γ 剂量率、环境样品)及食品、野生动植物等监测结果进行总结分析,并归纳综述近年来福岛地区环境及灾民健康情况。通过总结福岛第一核电站事故经验,结合我国国情进行了讨论分析。

【关键词】 福岛核事故; 放射性污染; 健康管理; 去污

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Situation and enlightenment in an environmental radioactivity and public health perspective seven years after Fukushima nuclear power plant accident

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【**Abstract**】 Since the accident on March 11th 2011 at the Fukushima Daiichi Nuclear Power Station following the Great East Japan Earthquake, huge amount of radionuclide has been released to the surrounding environment. In this study, the environmental monitoring method, γ-ray dose rates, radioactivity in environmental samples, food, wild animals and plants, health situation of residents were summarized. Through summarizing the accident experience of Fukushima Daiichi Nuclear Power Station, this research discussed and analyzed the accident combining with the situation in China.

【**Key words**】 Fukushima nuclear accident; Radioactive contamination; Health management; Decontamination

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2011 年 3 月 11 日 14:46,日本东北地区遭遇地震,50 min 后海啸来袭,21:23,政府发布福岛第一核电站 3 km 半径内避难指示;3 月 12 日 5:44 发布 10 km 半径内避难指示,15:36,1 号机组爆炸,18:25 发布 20 km 半径内避难指示;3 月 14 日 11:01,3 号机组爆炸,3 月 15 日,2 号机组合 4 号机组爆炸,发布半径 20~30 km 屋内避难指示。福岛核事故等级达到 7 级。地震时,约 78 000 人居住在核电站 20 km 范围内,62 000 人居住在 20~30 km 范围内。截至 2018 年 3 月 12 日,福岛县(相当于我国的省)死亡人数 4 051 人,损毁房屋 96 027 栋[1-2]。国际原子能辐射效应科学委员会(UNSCEAR)2013 年报告书推定向大气释放 ^{134}Cs(半衰期 2 年)为 9.0 PBq,^{137}Cs(半衰期 30 年)为 8.8 PBq,^{131}I(半衰期 8 d)为 120 PBq[3]。

我国迅速启动应急行动,对我国食品、饮用水、气溶胶样品进行监测及对日本入境人员进行表面污染检测,并及时与公众进行风险沟通[4]。事故后,对日本政府应急中的不足和经验教训,灾民照射水平和健康管理情况等进行了总结[5-6]。后福岛时期,张琼等[7]综述了日本灾后去污日程及方法,强调预先制定事故后恢复和治理的法规框架的重要性。各国应利用福岛事故的经验,改进和完善现有的核事故应急计划,并通过跟进其中长期的恢复过程,了解人工放射性核素的生态循环及对人群健康的危害。

目前,日本形成以政府机构与研究所、大学、医院、企业等机构合作的恢复模式。环境省(环境监测部门)、厚生劳动省(医疗卫生、社会保障的政府部门)、农林水产省(农作物

监测部门)、复兴厅及地方环境事务所等机构利用互联网持续更新数据。本文根据上述网站公布的数据(数据来源多为政府在各个地方设置的检查站和大学等学术机构)以及近年关于福岛地区的英文学术论文进行总结综述。

一、环境动态

事故前,福岛县环境 γ 剂量率本底范围为 0.07~0.12 μSv/h[8]。事故后,政府免费向居民出借 γ 剂量率检测仪器,并对学校、儿童活动区、景点、公共场所等特定场所进行集中监测。截至 2017 年 2 月,福岛县在公共场所设置固定 γ 剂量率监测仪器(探测地上 1 m)628 台;在儿童可能活动的场所设置适用于儿童的 γ 剂量率监测仪(探测地上 50 cm及1 m)3 099 台[9]。2017 年 4 月至 7 月,在居民返回区域,地上 1 m 高处环境 γ 剂量率的均值为(0.13±0.11)μSv/h、范围在 0.04~1.50 μSv/h 之间[10]。

除固定点位监测外,还使用直升机、移动车辆和步行相合的方式进行高密度、高覆盖、高频率的环境 γ 剂量率调查。直升机可在海上和地势复杂区域进行监测[11];车载剂量率仪器则用来测定污染范围及变化趋势[12];在居民结束避难返乡区域,利用步行调查寻找污染地区及估算居民在该地区生活的年剂量[13]。由于污染情况受天气及人类干预等因素影响,同一村庄、院落内污染程度也可能有很大不同[14-15],因此应进行长期监测,发现污染区域尽快去污,避免不必要的照射。

林业为福岛县主要产业(约 50%阔叶林,40%针叶林,剩余为竹林等),目前在树干、树液、新鲜落叶中均仍能发现较高浓度放射性铯[16]。通过修剪树枝、移除土壤等去污方式,2016 年 3 月,森林中环境 γ 剂量率大于 1 μSv/h 的区域减少到了 7%[17]。然而,Ayabe 等[18]指出,由于树叶的不断凋落,放射性铯仍在不断从树冠转移到地面,因此森林去污是无效的。有研究指出,放射性核素正逐渐向下层土壤迁移[19-20],且迁移速度快于切尔诺贝利地区[21-22]。目前,90%放射性铯主要存在于地表 10 cm 范围[19,23]。

截至 2017 年 9 月,日本参与去污人次达到 1 800 万人次,共除去土壤、废弃物合计 16 500 km^3。放射性废弃物首先放置于临时储存场,然后运送至中长期储藏地点,预计储存时间为 30 年[24]。

表 1 列出部分自然环境样品检测结果。在去污难度较大的河泥、湖泥中均检测到浓度较高的人工放射性核素,这同时会导致鱼类污染。地下水的污染目前尚不明显,但一些井水样品中检测到微量的放射性核素,因此持续检测仍旧是必要的。

二、水、食品及野生动植物监测结果

目前日本实行的食品标准为,放射性铯浓度(^{134}Cs+^{137}Cs)应低于:普通食品(100 Bq/kg)、牛奶(50 Bq/kg)、婴幼儿食品(50 Bq/kg)、饮用水(10 Bq/kg)[30]。近期该国政府公布的结果(表 2)可见,饮用水、大米、蔬菜、肉类、牛奶及栽培菌菇中的放射性活度均符合标准。但在野生动植物中检测到了较高的放射性铯活度。

有研究综述了福岛县菌菇样品结果,指出进入市场前的菌菇样品 2.7%~4.8%超出限值,进入市场后则为 0.6%~0.7%[39]。2015 年,川内村(事故 20 km 范围内)采集的野生菌菇中有 77.3%超出 100 Bq/kg,观测到^{134}Cs 活度的下降趋势,未观测到^{137}Cs 活度的下降趋势[35]。野生菌菇作为生态环境中的分解者,可以浓集大量放射性铯,因此在核事故后应仅在检测后进行选择性食用。

福岛县各乡镇中设有食品放射性核素无损检测设备,如居民带来的食物中放射性铯活度未超出限值,仍可带回家食用。同时使用高纯锗 γ 谱仪对居民提供的食品、饮用水等样品进行检测。对学校提供的食物进行每日抽检并在互联网公布结果。

三、人群健康与科普

2011 年 3 月 11 日,福岛县共有居民 2 024 401 人,事故发生后,共有 164 865 人离开家乡避难。截至 2019 年 1 月,仍有 42 104 人未返回家乡[2]。2017 年 4 月,浪江町、饭馆村、富冈町的部分区域解除避难指示和居住限制,居民开始返回[40]。然而,由于医疗服务、学校等公共服务设施不足、缺少就业岗位、担心辐射危害等原因,截至 2019 年 2 月,富冈町仅 864 人返回,占居民总数 6.6%[41]。

2011 年 6 月—2019 年 1 月,338 366 人进行了全身计数器检查[42-43],14 人>1 mSv,10 人>2 mSv,2 人>3 mSv。由于切尔诺贝利核事故后儿童甲状腺癌症发病率的上升[44],核事故后该地区展开了甲状腺疾病筛查工作。2011—2013 年

表 1 福岛县部分自然环境样品监测结果

Table 1 Radioactivity in environment samples from Fukushima prefecture

样品种类	时间	地点	样本数	^{134}Cs	^{137}Cs
河水	2017 年[25]	福岛县	326	ND	ND~0.75 Bq/L
河泥	2017 年[25]	福岛县	326	ND~720 Bq/kg	ND~6 000 Bq/ kg
湖水(水源地)	2017 年[25]	福岛县	306	ND~1.5 Bq/L	ND~15 Bq/L
湖泥	2017 年[25]	福岛县	210	ND~41 kBq/ kg	2.5~320 kBq/kg
湖泥	2014—2015 年[26]	福岛县真野大坝	25	28~40 kBq/kg(平均值范围)	
地下水	2018.5—2018.6[27]	福岛县	225	ND	ND
井水	2014—2016 年[28]	福岛县南相马市	11	-	ND~26.7 mBq/L
雨水	2018.4—2018.11[29]	福岛县福岛市	64	ND	ND~5.45 mBq/L

注:ND.小于探测下限;"-"为未测量

表 2　福岛地区部分饮用水、食品及野生动物调查结果

Table 2　Radioactivity in drinking water, food and wild animal samples from Fukushima prefecture

样品种类	日期	数量	$^{134}Cs+^{137}Cs$(Bq/kg)
饮用水[31]	2019.1	755	ND
大米[32]	2018.8—2019.3	9 175 336	<50
蔬菜[33]	2018.2—2019.2	1 454	ND ~40
肉类[33]	2018.2—2019.2	3 792	ND ~12.8
牛奶[33]	2018.2—2019.2	350	ND
海鱼[34]	2018.4—2019.2	6 326	ND ~220
栽培菌菇[33]	2018.2—2019.2	1 426	ND ~85.9
野生菌菇[35]	2015	159	ND ~5 600
养殖鱼[33]	2018.2—2019.2	7 444	ND ~195
无脊椎动物(蚯蚓)[36]	2017	33	3 400 ~19 000
两栖类(海龟)[36]	2017	3	1 900 ~11 000
鼠类[36]	2017	18	1 100 ~29 000
鸟类(燕子)[36]	2017	10	30 ~400
野猪[37]	2019	7	110 ~9 400
野猪[38]	2011—2016	1 033	900 ±2 743

注:ND.小于探测下限

度,对福岛县 300 473 人进行了甲状腺疾病筛查,乳头状癌 100 人;2014—2015 年度,筛查 270 511 人,甲状腺乳头状癌 43 人;2016—2017 年度,检查 191 669 人,甲状腺乳头状癌 7 人。目前为止福岛地区儿童甲状腺结节和囊肿发生率未见明显增加[45],若首次检查未发现甲状腺相关疾病,2 年或 5 年后再次筛查。

为应对灾民出现的抑郁、创伤后应激障碍、过度饮酒等问题[46],福岛县设置了 6 个心理疾病中心,7 个保健中心。定期进行健康讲座、心理咨询及健康体检等活动,并进行大量问卷调查,内容包括放射防护知识、家庭状况、心理健康、返乡意愿等[47-49]。调查显示,事故后居民的平均腰围增长 1 cm,体重增长 3 kg,代谢综合征、空腹血糖水平也有所增加[50]。

日本在事故后广泛面向群众开展辐射知识科普,放射知识科普读物区分为小学低年级,小学高年级及中学,中学生以上 3 个年龄段,并出版了英语版本[16];对核事故地区和核电站周边中小学教师进行辐射知识培训;设置电台、手机应用等进行辐射知识讲解;同时在每个村镇设置辐射防护相关知识咨询员等。

四、讨论

对比 2011 年,由于去污工作的持续进行及短半衰期核素的物理衰变,福岛地区居住环境中 γ 剂量率有了显著下降。根据其发布的监测结果,居民归还区域大部分已经恢复到事故前本底水平。在禁止进入区域,去污工作的持续进行使很多区域的解除封锁列入日程,但在中长期污染物贮存场,仍需要 30 年或更长的时间来恢复。食物和饮用水监测结果显示,居民通过本地食物摄入放射性核素的概率较低,不被认为会增加终生癌症发生率,但持续的食品监测是有必要的。

对于大部分区域被森林覆盖的福岛县,放射性铯在森林系统中较长生态半衰期导致了野生的动植物中人工放射性核素超过标准限值,发生核事故时应避免食用野生动植物,事故后应仅在检测并确认安全后食用。森林放射生态学、海洋放射生态学等与核事故恢复相结合的研究是必要的。

受灾民众逐渐选择结束避难返乡,与其各级机构采取的医疗保健、风险沟通方式等密切相关。医疗健康方面,对于事故时儿童群体,甲状腺疾病等仍需持续关注和队列研究。对于累积剂量较高的受灾群众,其后代的表观遗传学研究也是有必要的。通过对不同人群的放射防护知识普及和双向沟通,公众正在缓慢的恢复对权威机构信任。借鉴其经验,我国权威机构在风险沟通中应避免福岛事故应急阶段发生的专家口径不一致,如过度使用专有名词和使用不同辐射单位进行风险沟通等情况。后福岛时期,由于返乡居民较少,其权威机构和专家能够做到小组及深度访谈、入户检测、日常食品检测等。与大多数国家情况相同,我国灾后支援队伍中心理救援人员仍旧不足,因此对于应急人员的风险沟通培训和心理救援人员的辐射知识培训也是必要的。

我国人口较多且居住密集,核应急培训及演练难度较大。在互联网时代,通过社交网络对公众进行知识科普,可做到较强的覆盖广度。对中小学生进行辐射知识科普同样是值得参考的模式。需要注意的是,核事故时,电视、互联网、电话均可能无法使用,因此使公众熟悉避难地点位置、稳定性碘领取地点、服用时间及方式等非常重要。我们应当借鉴福岛事故经验,避免同样的错误,如将低剂量区域群众疏散到了高剂量地区、未能及时发放碘片等。近年来,各核电站积极开展核应急演练与培训,与国际组织合作和沟通,对公众开放核电站参观等,已取得积极的成效。与日本不同,我国核应急体系具备军民融合的特点,应当同时借鉴切尔诺贝利核事故的经验,加强应急救援人员辐射救援知识培训,避免辐射事故,保障公众和工作人员的安全。

利益冲突　本研究由署名作者按以下贡献声明独立开展,未接受有关公司的任何赞助,不涉及各相关方的利益冲突

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