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(please insert ref. above)

The Research Grants Council of Hong Kong
NSFC/RGC Joint Research Scheme
Joint Completion Report

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Fatty acid binding protein-4 as a mediator of autoimmune diabetes: from molecular mechanism to clinical significance

脂肪酸結合蛋白-4 誘發自身免疫糖尿病的作用機制及臨床意義

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Professor Aimin Xu	Professor Zhiguang Zhou
Post	Professor in Medicine, Professor in Pharmacology and Pharmacy, Director of State Key Laboratory of Pharmaceutical Biotechnology	Professor of Central South University, The Second Xiangya Hospital of Central South University
Unit / Department / Institution	Department of Medicine, The University of Hong Kong	The Second Xiangya Hospital of Central South University
Co-investigator(s) *(with title)*	Professor KSL Lam	Dr F Hu

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	01/01/2015		
Project Completion date	31/12/2018		
Duration *(in month)*	48 months		
Deadline for submission of Joint Completion Report	31/12/2019		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1) To investigate whether genetic ablation or pharmacological inhibition of FABP4 prevents or delay autoimmune destruction of pancreatic β cells, insulitis and overt diabetes in mice;
2) To evaluate the contribution of FABP4 to dynamic changes on infiltration and composition of immune cells in NOD mice;
3) To elucidate the molecular mechanisms by which FABP4 triggers insulitis and pancreatic β cell destruction by mediating immune cell crosstalk;
4) To explore the clinical relevance of FABP4 in the development of autoimmune diabetes in both Chinese and Caucasians

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

6. Research Outcome

Major findings and research outcome

(maximum 1 page; please make reference to Part C where necessary)

This joint research project enables us to conduct a comprehensive animal and clinical studies demonstrating that FABP4, a small lipid-binding protein, as an important etiological factor for development of type-1 diabetes (T1D). Our key findings are:

1. The expression of FABP4 in pancreatic islets and circulating level of FABP4 are progressively increased with the development of insulitis and T1D in NOD mice, which spontaneously develop autoimmune disease similar to humans. Both flow cytometry and immunocytochemistry analysis demonstrated that macrophages in the islets are the major site for increased FABP4 expression, whereas FABP4 is hardly detectable in dendritic cells, neutrophils, NK cells and adaptive immune cells.

2. Treatment of NOD mice with BMS309403, a cell- permeable, highly potent and selective inhibitor of FABP4, delays the onset time of diabetes from 13 weeks to 20 weeks of age, and also reduce the incidence of diabetes by 30% at 30 weeks of age. Furthermore, treatment with BMS309403 also alleviates insulitis and improves glucose tolerance in NOD mice. Likely, genetic ablation of FABP4 in NOD mice, by crossing FABP4 knockout mice with NOD mice for at least ten generations, also leads to a significant delay in diabetes onset time and a marked reduction in diabetes incidence, accompanied with alleviation of immune cell infiltration, insulitis, β-cell apoptosis, and decreases in expression of pro-inflammatory cytokines.
3. Genetic disruption of FABP4 obviously decreases the number of CD8+ cytotoxic T lymphocytes and shift the balance of CD4+ helper T lymphocytes (CD4+Th) from Th1 (IFNγ+CD4+) and Th17 (IL-17+CD4+) to Th2 (IL-4+CD4+) and Treg (Foxp3+CD4+) cells in pancreatic islets of NOD mice. Furthermore, FABP4-deficient NOD mice exhibit a significantly-reduced number of infiltrated macrophages, and an obvious shift from pro-inflammatory M1 ($F4/80^+CD11b^+CD11c^+$) to anti-inflammatory M2 ($F4/80^+CD11b^+CD206^+$) macrophages in pancreatic islets of NOD mice. Consistently, bone marrow-derived $FABP4^{-/-}$ macrophages exhibited a marked reduction of IFN γ/LPS-induced expression of iNOS (a marker for M1 macrophages), but an obvious elevation of IL4-induced expression of the M2 macrophage arginase, suggesting that FABP4 exerts its pro-inflammatory effects by promoting M2-to-M1 polarization. Macrophage depletion with Gdcl3 in NOD mice markedly delays the onset time from 12 weeks to 18 weeks, and also significantly reduces the incidence of diabetes from 86.7% to 62.3% at 30 weeks of age, accompanied with a marked reduction in insulitis score and in β-cell apoptosis, as determined by immunocytochemistry and TUNEL and biochemical analysis of caspase-3 activity in pancreatic islets. However, the effects of macrophage depletion on alleviation of insulitis, β-cell apoptosis and inflammatory cytokines in $FABP4^{-/-}$NOD mice are much less obvious than those observed in $FABP4^{+/+}$NOD mice. $FABP4^{+/+}$NOD mice transplanted with $FABP4^{-/-}$ bone marrow (BM) exhibit much lower incidence of diabetes than $FABP4^{+/+}$NOD mice transplanted with $FABP4^{+/+}$ BM, accompanied with significant reductions in insulitis , β-cell apoptosis, proinflammatory macrophages, autoreactive CD8 T cells, Th1 and Th17 but increased frequencies of Th2 and Treg cells. Taken together, these findings suggest that diabetogenic effect of FABP4 in NOD mice is at least in part attributed to its expression and actions in macrophages.
4. Mechanistically, FABP4 inhibits Janus Kinase (JAK)2 activity, thus impairing autophagy and exacerbating endoplasmic reticulum (ER) stress by decreasing toxic lipids-induced expression of autophagy-related protein Atg7, leading to pro-inflammatory responses and production of pro-inflammatory cytokines in macrophages.
5. Circulating levels of FABP4 is significantly elevated in patients with T1D and their first-degree relatives, and is closely associated with the titers of islet autoantibodies GADA ($r = 0.234$, $P = 0.002$) and IA2A ($r = 0.234$, $P = 0.002$) as well as the quantity of islet autoantibodies ($r = 0.219$, $P = 0.04$) in T1D patients.

The in vitro data described above has been published, and a manuscript on animal and clinical studies has been submitted and is currently under consideration (see pat C, section-8 for details.

<u>Potential for further development of the research and the proposed course of action</u> *(maximum half a page)*

Since our discovery of FABP4 as a pro-inflammatory biomarker associated with metabolic disease (*Xu A, Clin Chem. 2006 Mar;52(3):405-13,* cited >550 times), many pharmaceutical companies are developing selective chemical inhibitors of FABP4 as potential drugs for treatment of inflammatory and metabolic disease. The present study provides both genetic and

pharmacological evidence demonstrating that inhibition of FABP4 is a promising strategy for early intervention of T1D, an autoimmune disease associated with heightened inflammation. Based on our series of findings and the unique FABP4-deficient NOD mouse model as well as high throughput screening platforms generated in this study, we have established a strategic alliance with Servier (an European pharmaceutical company) to develop FABP4 chemical inhibitors for treatment of metabolic disease (secured a contract grant of ~ 5 million HKD). Furthermore, we have obtained an applied research grant from Guangdong-Hong Kong Technology Cooperation Funding Scheme of Hong Kong Innovation & Technology Council (GHP/079/18SZ) for development and preclinical Evaluation of FABP4 neutralizing monoclonal antibodies as potential therapeutic drugs, in collaboration with Prof. Junlei Chang at Shenzhen Institute of Advanced Technology, Chinese Academy of Science.

7. The Layman's Summary

(describe <u>in layman's language</u> the nature, significance and value of the research project, in no more than 200 words)

Type-1 diabetes (T1D) is the most severe form of diabetes occurring in childhood. There is currently no cure available for this disease, and T1D patients need to take lifelong injection with insulin. In this project, we have identified fatty acid binding protein-4 (FABP4) as an important driver for onset and progression of autoimmune destruction of insulin-producing β-cells in pancreatic islets, leading to T1D. Furthermore, we provided a series of evidence showing that genetic disruption or pharmacological inhibition of FABP4 is sufficient to delay the onset time and reduce the incidence of T1D in a mouse model with spontaneous development of autoimmune diabetes. Mechanistically, we uncovered a key role of FABP4 in activation of innate immune cells in pancreatic islets through its pro-inflammatory actions in macrophages, which in turn create a pro-inflammatory environment for cytotoxic T cells-mediated self-destruction of β-cells. In Chinese patients with T1D and their first-degree relatives, we found that circulating levels of FABP4 are elevated and closely associated with the titer of several islet autoantibodies for diagnosis of T1D. These findings not only help to understand the pathogenesis of T1D, but also support a crucial role of dysregulated innate immunity and metabolic inflammation in both T1D and type-2 diabetes. In light of the fact that chemical inhibitors of FABP4 are being developed for treatment of various inflammatory and metabolic diseases, our findings in the present study raise the possibility of the use of FABP4 chemical inhibitors for prevention and/or early therapeutic intervention of T1D and its complications.

<u>Part C: Research Output</u>

8. Peer-reviewed journal publication(s) arising <u>directly</u> from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Author(s)	Title and Journal/ Book	Submitted to RGC	Attached to this report	Acknowledged the support of this Joint Research Scheme	Accessible from the institutional repository
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*	***bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	*(with the volume, pages and other necessary publishing details specified)*	*(indicate the year ending of the relevant progress report)*	*(Yes or No)*	*(Yes or No)*	*(Yes or No)*

2017				Ruby L. C. Hoo*, Lingling Shu , Kenneth K. Y. Cheng , Xiaoping Wu, Boya Liao , Donghai Wu, **Zhiguang Zhou & Aimin Xu**	Adipocyte Fatty Acid Binding Protein Potentiates Toxic Lipids-Induced Endoplasmic Reticulum Stress in Macrophages via Inhibition of Janus Kinase 2-dependent Autophagy **Scientific Reports,** 2017 Jan 17;7:40657. doi: 10.1038/srep40657	2016	No	Yes	Yes
2020[@]		yes		Yang Xiao#, Lingling Shu#1, Xiaoping Wu, Lai Yee Cheong, Boya Liao, **Karen SL Lam**, Ruby LC Hoo, **Zhi-guang Zhou*, Aimin Xu***	Fatty Acid Binding Protein-4 Promotes Autoimmune Diabetes by Recruitment and Activation of Pancreatic Islet Macrophages Submitted to Diabetologia	no	yes	Yes	no

@: Submission of this manuscript was delayed due to difficulties in obtaining sufficient number of FABP4-deficient NOD mice, excessive long duration of each round of animal studies (~52 weeks per round), contamination of our animal units in certain stage, and the need to repeat the experiments.

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered

(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*

11/2015 Los Angles USA	Adipocyte fatty acid binding protein mediates adaptive thermogenesis via inducing intracellular thyroid hormone conversion	13th Annual World Congress on Insulin Resistance, Diabetes, and Cardiovascular Disease -WCIRDC	2016	No	Yes	Yes
11/2017 Singapore	FABP4 instigates type-1 diabetes by enhancing the crosstalk between innate and adaptive immune cells.	2017 Singapore Symposium on Metabolic Diseases	No	Yes	Yes	Yes
08/2018 Seoul, Korea	FABP4 mediates autoimmune destruction of beta cells by enhancing the crosstalk between innate and adaptive immunity	Asia Islet Biology and Incretin Symposium (AIBIS 2018)	No	Yes	Yes	Yes
03/2019 Hong Kong	FABP4 mediates autoimmune diabetes by enhancing the crosstalk between macrophages and tissue resident memory T cells	14th International Symposium on Healthy Aging, 2019	No	Yes	Yes	Yes

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Lingling Shu	PhD	01/09/2012	31/08/2016
Wang Xinmiao	Master of Medical Sciences	01/09/2016	31/8/2018

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

1. Collaboration: This project was conducted in close collaboration with Prof. Zhiguang Zhou's team at Xiangya Second Hospital, Central South University. The two teams have established

regular academic exchan.ge and joint supervision of research postgraduate students. We have also established a strategic alliance with Servier to develop antibody-based drugs for treatment of inflammatory and metabolic diseases by targeting FABP4.

2. Prizes: Dr. Lingling Shu (PhD student working on this joint project) has been awarded with **1st Runner-up Prize** for her oral presentation in 13th Annual World Congress on Insulin Resistance, Diabetes, and Cardiovascular Disease -WCIRDC, 2015, and **Best Abstract in Basic Science & Translational (Oral)** at 21st Medical Research Conference, Hong Kong, January 2019.

3. Patent award: Xu Aimin et al, Methods and Compositions for Use of Neutrophil Elastase and Proteinase 3 as Diagnostic Biomarkers**.** US P ATENT Number 9,625,460