CRF 8G (Revised Sep 15)

RGC Reference: HKUST10/CRF/12R
please insert ref. above

The Research Grants Council of Hong Kong
Collaborative Research Fund Group Research Projects
Completion Report

(*for completed projects only*)

Part A: The Project and Investigator(s)

1. Project Title

Protein Trafficking: Mechanism and Diseases

2. Investigator(s) and Academic Department/Units Involved *(please highlight approved changes in the composition of the project team and quote the date when RGC granted approval of such changes)*

Research Team	Name/Post	Unit/Department/Institution	Average number of hours per week spent on this project in the current reporting period
Project Coordinator	Jun Xia/Prof.	Life Science/HKUST	6
Co-Principal investigator(s)	Nancy Y. Ip/Chair Prof. Liwen Jiang/Prof. David K. Banfield/Prof. Jiandong Huang/Prof. Kenny K. Chung/Assoc Prof.	Life Science/HKUST Biology/CUHK Life Science/HKUST Biochemistry/HKU Life Science/HKUST	2 2 2 2 2
Collaborators/ Others			

3. Project Duration

	Original	Revised	Date of RGC Approval *(must be quoted*)
Project Start Date	Apr 1, 2013		
Project Completion Date	Mar 31, 2016		
Duration (*in month*)	36		
Deadline for Submission of Completion Report	Mar 31, 2017		

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Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. *Identify the transmembrane protein that binds to PICK1 on insulin granules and investigate its potential role in PICK1-mediated vesicle formation.*
2. *Explore the relationship of PICK1 and Kinesin-1 in vesicle transportation.*
3. *Investigate the role of Parkin in the function of PICK1-ICA69 protein complexes.*
4. *Elucidate the role of Cdk5 in the endophilin B1-mediated trafficking of NGF/TrkA and their potential implication in Alzheimer's disease.*

5.2 Revised objectives

Date of approval from the RGC: Mar 5, 2013

Reasons for the change: Following the suggestion from the panel member and reviewers, objective 4 was removed as it's not tightly related to other objectives.

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1. Identify the transmembrane protein that binds to PICK1 on insulin granules and investigate its potential role in PICK1-mediated vesicle formation.
2. Explore the relationship of PICK1 and Kinesin-1 in vesicle transportation.
3. Investigate the role of Parkin in the function of PICK1-ICA69 protein complexes.

6. Research Outcome

6.1 Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

In this project, we found that PICK1 and ICA69 formed heteromeric BAR-domain complexes that associated with insulin granules. While the PICK1-ICA69 heteromeric complexes associated with immature secretory granules, the PICK1-PICK1 homomeric complexes associated with mature secretory granules. PICK1 and ICA69 are important for insulin granule maturation. Deficiency in PICK1 led to the complete loss of ICA69, increased food and water intake but lower body weight in mice. Mice deficient in either PICK1 or ICA69 had high blood glucose due to insufficient insulin. More interestingly, we found that while mature insulin level was reduced in PICK1 knockout mice, proinsulin was increased. These data support the notion that PICK1 and ICA69 are key regulators of insulin granule maturation and their deficiency could contribute to the pathogenesis of diabetes.

We also generated conditional knockout mice of PICK1 that specifically eliminated PICK1 in the pancreatic beta-cells using the Cre-LoxP system. Five-month-old conditional Pick1 knockout mice exhibited impaired glucose tolerance, insulin insufficiency and hyperglycemia. In vitro experiments showed ablation of Pick1 in pancreatic beta cells selectively decreased the rapid initial release of insulin, as well as the total insulin in islets. Consistently, the immunostaining assay revealed that 40% of the Pick1 cKO islets had reduced insulin expression. Importantly, specific ablation of Pick1 induced elevated proinsulin in the circulating system and in the islet, which was accompanied by a reduction in proinsulin processing enzymes PC1/3 and PC2. Significantly, deletion of Pick1 triggered specific elimination of chromogranin B in pancreatic beta cells, which is believed to control granule formation and release. Furthermore, quantitative RT-PCR analysis results showed that proinsulin overproduction induced elevated expression of several UPR target genes, indicative of ER stress. Collectively, these data demonstrated a critical role of PICK1 in secretory granule biogenesis, proinsulin processing and beta cell function, and beta cells specific deletion of Pick1 in mice led to hyperglycemia and eventually diabetes.

We also studied the motor protein responsible for trafficking of PICK1-ICA69 positive vesicles. Our results indicate that PICK1-ICA69 positive vesicles were transported in a microtubule-dependent manner. In a search for the link between PICK1 and the transportation machinery, we found that PICK1 binds to Syntabulin, a kinesin binding protein mediating the synaptic vesicles' as well as mitochondria's trafficking in axons. We found that Syntabulin interacts with PICK1 and recruits PICK1 onto microtubule structures. We also demonstrated that Syntabulin mediates the trafficking of PICK1-containing vesicles along microtubules by time-lapse imaging. Syntabulin also forms a complex with PICK1 and neuronal receptors and regulates the complex trafficking via microtubules. In neurons, Syntabulin alters PICK1's expression by recruiting PICK1 into axons and regulates the trafficking dynamics of PICK1-containing vesicles, thereby mediating the expression of ASIC protein in neurons and participating in ASIC-induced acidotoxicity.

In term of Parkin's role in PICK1-ICA69-mediated protein trafficking, we confirmed the interaction between PICK1 and Parkin; a protein causes Parkinson's disease when mutated. This interaction was mainly mediated by the BAR domain of PICK1 and the RING1 domain of Parkin. PICK1 potently inhibited the E3 ligase activity of Parkin by disrupting its interaction with UbcH7. PICK1 promoted Parkin aggresome formation independent of proteasome inhibition. Parkin translocated to damaged mitochondria and led to their degradation in neurons, whereas PICK1 robustly inhibited this process. PICK1 also impaired the protective function of Parkin against stresses in SH-SY5Y cells and neurons. The protein levels of several Parkin substrates were reduced in young PICK1-knockout mice, and these mice were resistant to MPTP-mediated toxicity. Taken together, the results indicate that PICK1 is a potent inhibitor of Parkin, and the reduction in PICK1 enhances the protective effect of Parkin, which could be beneficial for Parkinson's disease treatment.

6.2 Potential for further development of the research and the proposed course of action *(maximum half a page)*

We are currently extending this line of research by investigating the potential role of PICK1 and ICA69 as key regulators of dense core vesicle biogenesis.

6.3 Research collaboration achieved *(please give details on the achievement and its relevant impact)*

The team collaborated in multiple levels, including student training, techniques and reagents exchanges, join meetings and symposiums. As a result, the team published two joint papers and has two more joint manuscripts under preparation (see part C).

7. The Layman's Summary

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

Our bodies are made of billions of cells with elaborate membrane compartments. Like in the logistics business, materials inside the cells must be transported between different cell compartments. The transportation of proteins between different cell compartments is called protein trafficking. Protein trafficking is critical to cells and abnormal trafficking of proteins causes many human diseases. In this study, we investigated the machineries that regulate protein trafficking, i.e., what controls the loading, transportation and unloading of cargos during protein trafficking. Specifically, we identified several key proteins that control the biogenesis of insulin granules and their deficiencies led to defective insulin secretion and other problems found in diabetes. We also obtained initial evidence that abnormal protein trafficking contributes to the pathogenesis of Parkinson's disease, a neurodegenerative disease.

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Part C: Research Output

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

(Please attach a copy of the 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) *(denote the corresponding author with an asterisk*)*	Title and Journal/Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of Acceptance (accepted but not published)	Under Review	Under Preparation (optional)						
		2017		He J, Yeung PKK, **Chung KK**, Chung SK and **Xia J***	PICK1 Inhibits the E3 Ubiquitin Ligase Activity of Parkin and Reduces Its Neuronal Protective Effect. *eLife*, under review	2017	No	Yes	
		2017		Li J, **Huang JD** and **Xia J***	PICK1 is essential for insulin production and maintenance of glucose homeostasis. *Mol. Cell. Biol.* In revision	2017	No	Yes	
	2017			Cui J, Pang J, Lin YJ, Gong H, Wang ZH, Li YX, Li J, Wang Z, Jiang P, Dai DP, Li J, Cai JP, **Huang JD***, Zhang TM*	Adipose-specific Deletion of Kif5b Exacerbates Obesity and Insulin Resistance in a Mouse Model of Diet-induced Obesity. *FASEB Journal*, in press	2017	Yes	Yes	Yes
2016				Cui J, Pang J, Lin YJ, Jiang P, Gong H, Wang Z, Li J, Cai JP, **Huang JD***, Zhang TM*	Conventional kinesin KIF5B mediates adiponectin secretion in 3T3-L1 adipocytes. *Biochemical and Biophysical Research Communications*. V476(4), P 620-626	2017	Yes	Yes	Yes
2016				Xu J, Wang N, Luo JH, **Xia J***	Syntabulin regulates the trafficking of PICK1-containing vesicles in neurons. *Scientific Reports* 6:20924	2017	Yes	Yes	Yes
2016				Wang H, Zhuang X, Wang X, Law AHY, Zhao T, Du S, Loy M, **Jiang L***	A Distinct Pathway for Polar Exocytosis in Plant Cell Wall Formation. *Plant Physiol.* 172(2):1003-1018	2017	Yes	Yes	Yes
2016				Chen L, Lau MS, **Banfield DK***	Multiple ER–Golgi SNARE transmembrane domains are dispensable for trafficking but required for SNARE recycling. Mol. Biol. Cell. 27(17):2633-41	2017	Yes	Yes	Yes

2015				Gao C, Zhuang X, Cui Y, Fu X, He Y, Zhao Q, Zeng Y, Shen J, Luo M and **Jiang L***	Dual roles of an Arabidopsis ESCRT component FREE1 in regulating vacuolar protein transport and autophagic degradation. *PNAS* 112(6):1886-1891	2017	Yes	Yes	Yes
2015				Wu W, Wan OW, **Chung KKK***	S-nitrosylation of XIAP at Cys 213 of BIR2 domain impairs XIAP's anti-caspase 3 activity and anti-apoptotic function. *Apoptosis.* 20:491-499	2017	Yes	Yes	Yes
2015				He J, Xia M, Tsang WH, Chow KL and **Xia J***	ICA1L forms BAR-domain complexes with PICK1 and is critical for acrosome formation in spermiogenesis. *J. Cell Sci.* 128(20):3822-36	2017	Yes	Yes	Yes
2015				Zeng Y, Chung KP, Li B, Lai CM, Lam SK, Wang X, Cui Y, Gao C, Luo M, Wong KB, Schekman R, and **Jiang L***	Unique COPII component AtSar1a/AtSec23a pair is required for the distinct function of protein ER export in Arabidopsis thaliana. *PNAS* 112(46):14360-5.	2017	Yes	Yes	Yes
2014				Xu J, Kam C, Luo J, **Xia J***	PICK1 Mediates Synaptic Recruitment of AMPA Receptors at Neurexin-induced Postsynaptic Sites. *J. Neurosci.* . 34(46):15415-15424	2014	Yes	Yes	Yes
2014				Ding Y, Wang J, Chun Lai JH, Ling Chan VH, Wang X, Cai Y, Tan X, Bao Y, **Xia J,** Robinson DG, **Jiang L***	Exo70E2 is essential for exocyst subunit recruitment and EXPO formation in both plants and animals. *Mol. Biol. Cell.* 25(3):412-26	2014	No	Yes	Yes
2013				Cao M, Mao Z, Kam C, Xiao N, Cao X, Shen C, Cheng KK, Xu A, Lee KM, **Jiang L, Xia J*.**	PICK1 and ICA69 control insulin granule trafficking and their deficiencies lead to impaired glucose tolerance. *PLoS Biology.* 11(4):e1001541	2014	No	Yes	Yes
2013				Wang H, Zhuang XH, Cai Y, Cheung AY and **Jiang L***.	Apical F-actin-regulated exocytic targeting of NtPPME1 is essential for construction and rigidity of the pollen tube cell wall. *The Plant Journal* 76(3): 367-379.	2014	No	Yes	Yes
2013				Zhuang X, Wang H, Lam SK, Gao C, Wang X, Cai Y and **Jiang L***	A BAR-Domain Protein SH3P2, Which Binds to Phosphatidylinositol 3-Phosphate and ATG8, Regulates Autophagosome Formation in Arabidopsis. *The Plant Cell* 25: 4596-4615.	2014	No	Yes	Yes
2013				Li X, Mao Z, Wu M, **Xia J***	Rescuing infertility of Pick1 knockout mice by generating testis-specific transgenic mice via testicular infection. *Sci. Rep. 8;3:2842*	2014	No	Yes	Yes

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each conference abstract)*

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 RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
7/2013/Singapore	PICK1 Regulates Dense Core Vesicle Trafficking	International Conference on Neuron and Brain Disorders	2014	Yes	Yes	No
9/2014/Yokohama, Japan	Roles of PICK1-ICA69 BAR Domain Complexes in Protein Trafficking	Japanese Neuroscience Meeting	2014	Yes	Yes	No

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

N/A

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