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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

The role of the BMP co-receptor Dragon in kidney tubular development and regeneration

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. XIA Yin	Prof. Baoxue YANG
Post	Assistant Professor	Professor
Unit / Department / Institution	School of Biomedical Sciences/The Chinese University of Hong Kong	Department of Pharmacology/School of Basic Medical Sciences/Beijing University
Contact Information	Xia.Yin@cuhk.edu.hk 3943 4480	baoxue@bjmu.edu.cn
Co-investigator(s) *(with title and institution)*		Dr. Hong ZHOU, Associate Professor/Department of Pharmacology/School of Basic Medical Sciences/Beijing University

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	1/1/2013		
Project Completion date	31/12/2016		
Duration *(in month)*	48 months		

Deadline for Submission of Completion Report	31/12/2017		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. *Determine the roles of Dragon and the underlying mechanisms in embryonic renal branching morphogenesis in vivo.*
2. *Study the roles of Dragon in renal tubular injury and repair in adult kidneys.*
3. *Examine the mechanisms of the action of Dragon in tubulogenesis in vitro.*
4. *Elucidate the mechanisms by which Dragon enhances the utilization of ActRIIA by BMP4.*

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

1.
2.
3. ....

6. Research Outcome

Major findings and research outcome

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

As proposed in Objectives 1 and 3, we studied the role and the underlying mechanisms of action of Dragon in embryonic kidney development. We found that Dragon was highly expressed in renal tubular epithelial cells in embryonic kidneys. Dragon-null E13.5 kidneys had apparently fewer and thicker UB branches than wild-type ones. Consistently, kidneys from Dragon-null mice at the postnatal ages of 10 and 14 days showed reduced number of collecting ducts compared to wild-type ones. These results suggest that Dragon plays an important role in renal tubular development. Unexpectedly, we found that Dragon inhibited renal cyst development as shown by cultured wild-type and Dragon-null embryonic kidneys and MDCK cyst model. Dragon's activity in branching morphogenesis and cyst development was mediated by the BMP/Smad pathway. This work was mostly done by Professor Baoxue YANG, the PI in Mainland China, and has been reported in Cellular Signaling (2016).

As proposed in Objective 2, we have studied the role of Dragon in kidney injury and repair using our heterozygous Dragon knockout mice. We found that Dragon inhibited E-cadherin expression and induced hypoxia-induced apoptosis in kidney epithelial cells in vitro. Compared with wild-type mice, heterozygous Dragon knockout mice exhibited decreased epithelial apoptosis, and increased tubular E-cadherin expression and had attenuated tubular injury after unilateral ureteral obstruction (UUO). Our results suggest that Dragon may impair tubular epithelial integrity and induce epithelial apoptosis both in vitro and in vivo. We also found that the action of Dragon on E-cadherin expression and apoptosis in renal epithelial cells was mediated through the through the neogenin pathway but not the BMP pathway (Objective 4). These observations led us to switch from the BMP pathway to the neogenin pathway. Due to failure of Dragon to affect Cisplatin-induced cell death in vitro, we did not continue to study the role of Dragon in Cisplatin-induced acute kidney injury. This work was mostly done by Prof. Yin XIA, the PI in Hong Kong, and has been reported in J Biol Chem (2013).

The stimulatory effects of Dragon on apoptosis was not observed in renal ischemia/reperfusion injury (IRI) either. To further identify the specific role of Dragon in renal tubular cells during acute kidney injury (AKI), we generated renal tubular cell specific Dragon knockout mice. Since emerging evidence indicates that necroptosis plays a critical role in the development of AKI, we studied the role of Dragon in necroptosis. Necroptosis is executed by mixed lineage kinase domain-like protein (MLKL) upon its binding to the plasma membrane. We found that Dragon reduced membrane-associated MLKL levels and inhibited necroptosis in cultured cells. During IRI or oxalate nephropathy, MLKL was induced to express on the apical membrane of proximal tubular (PT) cells. Specific knockout of Dragon in tubular cells increased MLKL expression at the apical membrane of PT cells and induced more tubular cell death and more severe renal function impairment when compared with wild-type mice. Treatment with the necroptosis inhibitor Necrostatin-1 or GSK'963 reduced MLKL expression on the apical membrane of PT cells and ameliorated renal dysfunction after IRI in both wild-type and Rgmb cKO mice. Taken together, our results suggest that RGMb protects against AKI by inhibiting MLKL membrane association and necroptosis in proximal tubular cells. This work is most done by Prof. XIA's group. It is an extension of the project. The findings are novel and exciting, and received very positive comments from PNAS with minor revisions. Now the revised version has been resubmitted to PNAS.

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

1. Autosomal dominant polycystic kidney disease (ADPKD) is a monogenetic disease that still lacks effective therapy. It commonly causes end-stage renal failure and affects approximately 1/1000-1/400 of individuals. Our finding that Dragon inhibits renal cyst formation is very important, but these results need to be confirmed. We would propose to crossbreed our floxed Dragon mice with Pkd2 knockout mice to see if deletion of Dragon delays renal cyst formation and development in Pkd2 knockout mice.
2. Our finding on the role of Dragon in necroptosis opens a new arena of the study on necroptosis. We will continue to further investigate how Dragon reduces MLKL membrane association, and whether soluble Dragon proteins inhibit necropsosis and ameliorate acute kidney injury.

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)

Through this project, we found that Dragon is required for normal branch formation in embryonic kidneys. Dragon inhibits renal cyst development via the BMP signaling pathway under certain pathological conditions. Dragon induces apoptosis and inhibits necroptosis, and plays different roles in the pathogenesis of different types of kidney injury.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly 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)*

2013				Wenjing Liu, Xiaoling Li, Yueshui Zhao, Xiao-Ming Meng, Chao Wan, **Baoxue Yang**, Hui-Yao Lan, Herbert Y. Lin*, and **Yin Xia***	Dragon (repulsive guidance molecule RGMb) inhibits E-cadherin expression and induces apoptosis in renal tubular epithelial cells. **J Biol Chem.** 288(44):31528-39.	Yes 31/12/2014	Yes	Yes	Yes
2014				Wu XG, Wang Y, Wu Q, Cheng WH, Liu W, Zhao Y, Mayeur C, Schmidt PJ, Yu PB, Wang F, **Xia Y***.	HFE interacts with the BMP type I receptor ALK3 to regulate hepcidin expression. **Blood.** 21;124(8):1335-43.	Yes 31/12/2014	Yes	Yes	Yes
2015				Shi Y, Chen GB, Huang XX, Xiao CX, Wang HH, Li YS, Zhang JF, Li S, **Xia Y***, Ren JL*, Guleng B*.	Dragon (repulsive guidance molecule b, RGMb) is a novel gene that promotes colorectal cancer growth. **Oncotarget**. 6(24):20540-54.	No	Yes	Yes	Yes
2015				Wang C, Kam RK, Shi W, **Xia Y**, Chen X, Cao Y, Sun J, Du Y, Lu G, Chen Z, Chan WY, Chan SO, Deng Y, Zhao H.	The Proto-oncogene Transcription Factor Ets1 Regulates Neural Crest Development through Histone Deacetylase 1 to Mediate Output of Bone Morphogenetic Protein Signaling. **J Biol Chem**. 290(36):21925-38.	No	No	Yes	Yes
2016				Shi Y*, Huang XX, Chen GB, Wang Y, Zhi Q, Liu YS, Wu XL, Wang LF, Yang B, Xiao CX, Xing HQ, Ren JL, **Xia Y***, Guleng B*.	Dragon (RGMb) induces oxaliplatin resistance in colon cancer cells. **Oncotarget.** 7(30):48027-48037.	No	Yes	Yes	Yes
2017				Liu J, Wang W, Liu M, Su L, Zhou H, **Xia Y**, Ran J, Lin HY, **Yang B**.	Repulsive guidance molecule b inhibits renal cyst development through the bone morphogenetic protein signaling pathway. **Cell Signal.** 28(12):1842-1851.	No	Yes	Yes	Yes
	2017 (accepted)			Huihui Huang; Chunhua Xu; Yang Wang; Wenjing Liu; Yueshui Zhao; Xiao-Ru Huang; Wenxing You; Bo Feng; Zhi-Hua Zheng; Yu Huang; Hui-Yao Lan; Jinzhong Qin*; **Yin Xia***.	L3MBTL2 protects against kidney injury by inhibiting the DNA damage-p53-apoptosis pathway in renal tubular cells. **Kidney International**.	No	Yes (article in press)	Yes	Yes
		2017 (in minor revision)		Wenjing Liu, Binbin Chen, Yang Wang, Chenling Meng, Huihui Huang, Xiao-Ru Huang, Jinzhong Qin, Shrikant R. Mulay,	RGMb protects against acute kidney injury by inhibiting tubular cell necroptosis via an MLKL-dependent				

				Hans-Joachim Anders, Andong Qiu, **Baoxue Yang**, Gordon J. Freeman, Hua Jenny Lu, Herbert Y. Lin, Zhi-Hua Zheng, Hui-Yao Lan, Yu Huang, **Yin Xia***	mechanism. **Proc Natl Acad Sci U S A**.	No	Yes (submitted revised manuscript)	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 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)*
04/2016/ Hyogo, Japan	An update on the biological roles of RGMb in the kidney and gonad	The 1st Repulsive guidance molecule international symposium	No	Yes	No	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
Wenjing Liu	PhD in Biomedical Sciences	August, 2011	June 2014/August 2014
Huihui Huang	PhD in Biomedical Sciences	August, 2013	June 2016/August 2016
Chenling Meng	PhD in Biomedical Sciences	August, 2014	June 2017/August 2017

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

1. Best Poster Award awarded by China Society of Renal Physiology to Wenjing Liu (2013-05)
2. The third best award for oral presentations awarded by Guangdong-Hong Kong-Macau Postgraduate Symposium on Regenerative Medicine 2013 to Wenjing Liu (2013-11)
3. The Second Best Award for Oral Presentation awarded by Guangdong-Hong Kong Postgraduate Symposium on Regenerative Medicine to Huihui Huang (2014-12).
4. The Third Best Award for Oral Presentation awarded by Guangdong-Hong Kong Postgraduate Symposium on Regenerative Medicine to Chenling Meng (2016-12).
5. The Thirst Best Award for Poster Presentation Guangdong-Hong Kong Postgraduate Symposium on Regenerative Medicine to Binbin Chen (2016-12).
6. The Award of Nomination, SBS Postgraduate Research Day 2017, organized by the Postgraduate Student Association of the School of Biomedical Sciences, The Chinese University of Hong Kong, to Chunhua Xu (2017-11).
7. The Second Best Award for Oral Presentation awarded by Guangdong-Hong Kong Postgraduate Symposium on Regenerative Medicine to Chunhua Xu (2017-12).