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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

Molecular mechanism of notochord formation regulated by XBP1

XBP1 調節脊索形成的分子機制研究

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Dr. Hui Zhao	Dr. Cao Ying
Post	Assistant Professor	Professor
Unit / Department / Institution	School of Biomedical Sciences/Faculty of Medicine/The Chinese University of Hong Kong	Model Animal Research Centre, Nanjing University
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Co-investigator(s) *(with title and institution)*	Prof. Chan Wood Yee School of Biomedical Sciences/Faculty of Medicine/The Chinese University of Hong Kong	

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	01/01/2013	N. A.	
Project Completion date	31/12/2016	N. A.	
Duration *(in month)*	48	N. A.	
Deadline for Submission of Completion Report	31/12/2017	N. A.	

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. Investigate the transcriptional regulation of Sec23 by XBP1 during notochord formation.
2. Investigate molecular mechanisms of XBP1 nuclear translocation.
3. Investigate interactions between XBP1 and Grp78/bip during notochord formation.

5.2 Revised Objectives

Date of approval from the RGC: N. A.

Reasons for the change: N. A.

6. Research Outcome

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

1) We have established gene disruption approaches such as TALEN and CRISPR, and utilized the approaches to establish *Xenopus* Hspa5 and Rrbp1 knockout line, which set up a base to study the function of XBP1 and Hsap5a by using genetic approaches. We now obtained the Hspa5^{+-} frogs harbouring seven-nucleotide deletion. We are now expanding the colony of Hspa5$^{+/-}$ *X. tropicalis, and* will perform RNA-Seq to identify the downstream genes mediated by Hspa5. Xbp1 sgRNA was designed, and was proved effective to disrupt the Xbp1 gene in *X. tropicalis* in trial experiments. We will utilize this sgRNA to establish the Xbp1-/- line in the future study.

2) We examined the expression of *Xbp1* and *Hspa5* in developing *X. laeivs* embryos with whole mount in situ hybridization. The spatial expression pattern of Xbp1 and Hsap5a is quite similar and largely overlapped. Indeed, overexpress *Xbp1* can induce up-regulation of *Hspa5* in *Xenopus* embryos. Knockdown of Hspa5 can moderately increases the expression of unspliced *Xbp1*, but strongly inhibits the expression of notochord marker *Chordin* and *Not*, and mesoderm marker, *bra*.

3) We found that Xbp1 and P85α can physically interact each in *Xenopus* embryos. Co-injection of Xbp1 and P85α promotes the expression of *fgf8* when compared to single injection of *Xbp1*. Xbp1 can promote the gene expression of *Hspa5*, *Rrbp1*, *CopA*, *DnaJB9*, *Hsp90B1*, *Sec61g* and *Tloc*, which have been implicated in ER stress or protein secretion. However, the co-injection of Xbp1 and P85α does not further increase the expression of these genes.

4) We used morpholino antisense oligonucleotides (MO) to knockdown endogenous Hspa5 in *Xenopus* embryos successfully. Global knockdown of Hspa5 caused severe gastrulation defect, showing open blastopore and inhibition of convergent extension. When injected at the low dose, the Hspa5MO reduced the expression of *Chordin*, *bra* and *Not*, the notochord markers. These results indicate that Hsap5a is required for notochord formation.

5) We found Hspa5 is required for the transduction of retinoic acid (RA) signalling. Knockdown of Hspa5 does not affect the transcription of genes involved in RA signalling transduction and atRA metabolism including *RARα*, *RXRα*, *RXRβ*, *Raldh1*, *Raldh2*, and *Raldh3*. However, the protein level of retinoic acid receptor, RARα protein is much

reduced upon knockdown of Hspa5. Thus, our data suggest that Hspa5 regulates retinoic acid signalling, at least in part, by mediating the expression level of RARα.

6) In Hspa5 morphants, pronephros formation was strongly inhibited. Pronephros tissue is induced *in vitro* by treating animal caps with *all-trans* retinoic acid (atRA) and activin. Depletion of Hspa5 in animal caps, however, blocked the induction of pronephros as well as reduced the expression of RA-responsive genes, suggesting that knockdown of Hspa5 attenuated RA signalling. Knockdown of Hspa5 in animal caps resulted in decreased expression of *lhx1,* a transcription factor directly regulated by RA signalling and essential for pronephros specification. Co-injection of Hspa5MO with *lhx1*mRNA partially rescues the phenotype induced by Hspa5MO. These results suggest that the RA-lhx1 signalling cascade is involved in Hspa5MO induced pronephros malformation. This study shows that Hspa5, a key regulator of the unfolded protein response, mediates RA signalling during early embryonic development.

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

We have obtained F1 Hspa5$^{+/-}$frogs harbouring seven-nucleotide deletion. We next want to collect the Hspa5$^{-/-}$ embryos, and use RNA-seq to identify the gene mediated by Hspa5. We found that depletion of human HSPA5 induces down-regulation of RARα at protein level in cultured SH-SY5Y cells. Yet the underlying mechanism remains unknown. We plan to investigate whether knockdown of HSPA5 will affect other retinoic acid receptors, and whether knockdown of HSPA5 will affect the ubiquitination of RARα.

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)

During the embryonic development, the increase of protein synthesis causes an accumulation of unfolded proteins in the endoplasmic reticulum (ER), a stress condition so-called ER stress, revealed by up-regulation of Xbp1 and Hspa5. We found the two genes highly expressed in developing embryos, displaying specific and overlapping pattern. XBP1 can also up-regulate Hspa5 expression. The functions of XBP1 and Hspa5 in embryonic development are largely unknown. Our study makes the efforts to delineate the roles of Xbp1and Hspa5 during embryonic development, and provide more insights into regulatory networks in tissue patterning and further our understanding on physiological roles of ER stress during embryonic development. We report for the first time that Hspa5 regulates retinoic acid signalling, and is required for the maintaining the expression of retinoic acid (RA) receptor, RARα, at protein level. Given the facts that ER stress and RA signalling are involved in the immune response, diabetes, tumor growth under hypoxic conditions, and neurodegenerative diseases. Our research will also be helpful to develop new therapeutic strategies for the treatment of these diseases.

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) *(**bold** the authors belonging to the project teams and 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 this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*						
2016				Wong CB, Rebbert M, Wang CD, Chen XF, Heffer A, Zarelli VE, Dawid IB, and **Zhao H.***	Genes regulated by potassium channel tetramerization domain containing 15 (Kctd15) in the developing neural crest. ***Int. J. Dev. Biol.*** (2016), 60:159-166**.**	No	Yes	Yes	Yes
2016				Liu ZZ, Cheng TTK, Shi ZY, Liu ZR, Lei Y, Wang CD, Shi WL, Chen XF, Qi XF, Cai DQ, Feng B, Deng Y, Chen YL, and **Zhao H.***	Efficient genome editing of genes involved in neural crest development using the CRISPR/Cas 9 system in *Xenopus* embryos. ***Cell and Bioscience***. (2016), 6: 22, s13578-016-0088-4.	No	Yes	Yes	Yes

2015				Shi Z, Wang F, Cui Y, Liu Z, Guo X, Zhang Y, Deng Y, **Zhao H***, and Chen Y.	Heritable CRISPR/Cas 9-mediated targeted integration in *Xenopus tropicalis*. 2015 (12):4914-49 23.	2014	Yes	Yes	Yes
2015				Shi WL, Xu G, Wang CD, Sperber SM, Chen YL, Zhou Q, Deng Y, and **Zhao H***	Heat shock 70-kDa protein 5 (Hspa5) is essential for pronephros formation by mediating retinoic acid signaling. ***J Biol Chem*** 2015, 290: 577-589**.**	2014	Yes	Yes	Yes

2015				Wang CD, Kam RTK, Shi WL, Xia Y, Chen XF, **Cao Y**, Sun J, Du Y, Lu G, Chen ZJ, Chan WY, Chan SO, Deng Y, and **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*** 2015, 290: 21925-21938.	No	Yes	Yes	Yes
2014				Liu Y, Luo DY, Lei Y, Hu W, **Zhao H***, and Cheng CHK.	A highly effective TALEN-mediated approach for targeted gene disruption in *Xenopus tropicalis* and zebrafish ***Methods* (2014)** S1046-2023 (14) 00041-3	2014	Yes	Yes	Yes
2013				Kam RKT, Shi W, Chan SO, Chen Y, Xu G, Lau CBS, Fung KP, **Chan WY***, **Zhao H**.*	dhrs3 attenuates the retinoic acid signaling and is required for early embryonic patterning. ***J Biol Chem*** (2013) 288: 31477-31487.	2014	Yes	Yes	Yes

2013				Lei Y, Guo X, Deng Y, Chen YL, **Zhao H***.	Generation of gene disruptions by transcription activator-like effector nucleases (TALENs) in *Xenopus tropicalis* embryos ***Cell & Bioscience*** 3, 21.	2014	Yes	Yes	Yes
2015				Lu L, Gao Y, Zhang Z, Cao Q, Zhang X, Zou J, **Cao Y*.**	Kdm2a/b Lysine Demethylases Regulate Canonical Wnt Signaling by Modulating the Stability of Nuclear β-Catenin.***Dev Cell***. 2015 22;33(6):660-74.	No	Yes	Yes	Yes
2015				Zhang X, Gao Y, Lu L, Zhang Z, Gan S, Xu L, Lei A, **Cao Y***.	JmjC Domain-containing Protein 6 (Jmjd6) Derepresses the Transcriptional Repressor Transcription Factor 7-like 1 (Tcf7l1) and Is Required for Body Axis Patterning during Xenopus Embryogenesis. ***J Biol Chem***. 2015 290(33):20273-20283.	No	Yes	Yes	Yes

2015				Gao Y, Cao Q, Lu L, Zhang X, Zhang Z, Dong X, Jia W, and **Cao Y**.	Kruppel-like factor family genes are expressed during Xenopus embryogenesis and involved in germ layer formation and body axis patterning. ***Dev Dyn***. 2015 244(10): 1328-46.	No	Yes	Yes	Yes
2015				Cao Y.*	Germ layer formation during Xenopus embryogenesis: the balance between pluripotency and differentiation. ***SCIENCE CHINA Life Sciences*** 2015, 58 (4), 336-342.	No	Yes	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)*
8/2014 Pacific Groove, California, USA	Heat shock 70kDa protein 5 (Hspa5) is essential for pronephros formation by mediating retinoic acid signaling". (Poster presentation)	The 14th International Xenopus Conference	Yes	Yes	Yes	No.
March 2014, Hiroshima University, Japan	Efficient targeted gene disruption in Xenopus embryos using TALEN and Crispr/Cas9 systems (Oral presentation)	International symposium "Frontiers in Amphibian Biology: Endangered Species Conservation and Genome Editing	Yes	Yes	Yes	No.

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
Shi Weili	Ph. D	08/2010	01/2014
Lei Yong	Ph. D	08/2010	07/2013
Wang Chengdong	Ph. D.	08/2010	07/2013
Liu Zhongzhen	Ph. D.	8/2012	07/2015
Zhao Xin	MPhil	8/2013	11/2015
Cheng Tsz Kwan	MPhil	8/2012	10/2014

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

N. A.