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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

Role of TAM receptor tyrosine kinases on blood-testis barrier function and testicular innate immunity

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof Will M Lee	Prof Daishu Han
Post	professor	professor
Unit / Department / Institution	School of Biological Sciences University of Hong Kong	Department of Cell Biology Basic Institute of Medical Sciences Chinese Academy of Medical Sciences Beijing Union Medical College
Co-investigator(s) *(with title)*	NA	NA
Others	Dr. Mok KW (postdoc) Dr. Xiao Xiang (postdoc) Dr. Li Nan (postdoc) Dr. Gao Y (postdoc) Dr. Wen WQ (postdoc) Miss Tang EI (PhD student) Mr Chen HQ (PhD student)	

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	1-1-2013	no	
Project Completion date	31-12-2016	no	

Duration *(in month)*	48	no	
Deadline for submission of Joint Completion Report	31-12-2017	no	

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

Hong Kong Team :

To examine the role of receptor tyrosine kinases signaling molecules in maintaining blood-testis barrier (BTB) integrity by assessing the mechanism underlying changes in cell adhesion at the BTB and Sertoli-germ cell interface using animal models and cell cultures..

Mainland team

To examine the role of receptor tyrosine kinases on testicular innate immunity.

5.2 Revised Objectives

N.A.

6. Research Outcome

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

We have critically evaluated findings based on studies at the BTB in rodents, illustrating that mTORC1 [10, 27] and p-FAK-Tyr397 [1, 26] signaling complexes and their corresponding signaling pathways promote BTB remodeling, leading to the immunological barrier to become "leaky". However, mTORC2 and p-FAK-Tyr407 signaling complexes and their corresponding pathways promote BTB integrity, making the barrier "tighter". These two pairs of signaling proteins, namely mTORC1 vs. mTORC2, and p-FAK-Tyr397 vs. p-FAK- Tyr407, have antagonistic effects on the BTB integrity, by serving molecular switches to turn "off" or "on" the immunological barrier, thereby supporting the transport of preleptotene spermatocytes across the barrier during spermatogenesis. It is also likely that p-FAKs are working in concert with the c-Src/c-Yes non-receptor protein kinases which are recently shown to play a role in modulating BTB dynamics [5] since FAK and Src are signaling partners known to regulate mammalian cell physiology. More important, the respective up-stream molecule(s) that trigger either mTORC1/mTORC2 and/or p-FAK-Tyr397/p-FAK- Tyr407 activation is not fully elucidated. However, recent studies have suggested that the local regulatory axis that connects the basement membrane and the BTB [21] may be utilizing mTOR to modulate basal ES/BTB function. For instance, laminin α2 chain in the basement membrane adjacent to the BTB, possibly through the 80 kDa fragment from its C-terminus, was found to promote the BTB function, making the BTB tighter, since the knockdown of laminin α2 by RNAi using laminin α2-specific shRNA (small hairpin RNA) vs. control nontargeting shRNA was shown to perturb the Sertoli cell TJ-barrier function via its disruptive effects on F-actin organization in Sertoli cells [21, 22]. More important, the laminin α2 knockdown was associated with an up-regulation of p-rpS6 expression [22], and rpS6 is the downstream signaling molecule of mTORC1 which is known to promote BTB disruption as discussed herein. Furthermore, the use of rapamycin, a specific inhibitor of mTORC1, was found to block the laminin α2 shRNA-mediated disruptive effects on F-actin organization in Sertoli cells [22]. Collectively, these recent findings support the notion that laminin α2 chain and/or its biologically active fragment(s) may be the upstream regulatory of the mTORC1/mTORC2 regulatory signaling molecules to modulate BTB dynamics.

On the other hand, the local functional axis that connects the apical ES and the BTB may be utilizing FAK to regulate BTB function. It has been shown that F5-peptide, a biologically active 50-amino acid res- idue fragment generated from the laminin-γ3 chain at the apical ES (i.e., at the Sertoli-late spermatid interface) at stage VIII of the cycle, exerts its effects by perturbing the spatiotemporal expression of p-FAK-Tyr407 at the apical and basal ES/BTB, associated with gross disruption on the organization of F-actin network across the seminiferous epithelium. These changes in turn perturbed adhesion function at the apical and basal ES, leading to spermatid exfoliation and BTB disruption based on studies in vitro and in vivo [20]. In fact, overexpression of the p-FAK-Tyr407 phosphomimetic (and constitutively active) mutant p-FAK-Y407E was shown to block the disruptive effects of F5-peptide on Sertoli cell TJ-barrier

function. In short, the laminin α2 chain and the F5-peptide derived from laminin γ3 may be the corresponding upstream molecule that triggers the mTORC1/mTORC2 and p-FAK- Tyr397/p-FAK-Tyr407 activation and/or inactivation of these signaling molecules. It is obvious that much work is needed to further expand these findings. Nonetheless, it is likely that germ cells, such as preleptotene sper- matocytes in the basal compartment and elongated spermatids in the adluminal (apical) compartment are playing an important role by serving as the upstream regulators to modulate BTB dynamics, such as through the production of laminin α2 chain-derived peptide(s) in the basement membrane [21, 22, 24] or F5-peptide from the laminin-γ3 chain at the apical ES [20] as briefly discussed above. On the other hand, proteins such as Formin 1, connexin 43, actin bundling proteins that are expressed at the apical ES and TJ also modulate the barrier function as recently examined in this study [3,7,9,11,12,15,18]. Emerging evidence has also supported the involvement of polarity proteins BTB formation and maintenance [14,17,19,25]. Collectively, the findings obtained herein have supported our beliefs that the hypothetical model depicted in the beginning paragraph and it provides the framework for investigators to design functional experiments in future years to study BTB biology, and to assess the applicability of this information in studying other blood–tissue barrier.

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

Our understanding of the role of signaling molecules in maintaining blood-testis barrier (BTB) integrity has led us to the use of overexpressing p-FAK-Y407E or inhibitor of Akt1 to alleviate toxidant-induced Sertoli cell injury in rodents and humans [1, 23, 26]. This information, if deciphered and better understood, will provide better therapeutic management of diseases particularly in organs that are sealed by the corresponding blood–tissue barriers from systemic circulation, such as the brain and the testis [13].

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)

The current study provided significant insights to the understanding of signaling pathways that regulate blood–tissue barriers, and for studying the biology of various blood– tissue barriers to a larger extent. This information, if deciphered and better understood, will provide better therapeutic management of diseases particularly in organs that are sealed by the corresponding blood–tissue barriers from systemic circulation, such as the brain and the testis. These barriers block the access of antibiotics and/or chemotherapeutical agents across the corresponding barriers. Studies in the last decade using the blood–testis barrier (BTB) in rats have demonstrated the presence of several signaling pathways that are crucial to modulate BTB function. Herein, we critically evaluate these findings and provide hypothetical models regarding the underlying mechanisms by which these signaling molecules/pathways modulate BTB dynamics. This information should be carefully evaluated to examine their applicability in other tissue barriers which shall benefit future functional studies in the field.

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.)

Publications #1-8 were reported and submitted in the progress report in 2015.

1. Wan HT, Mruk DD, Li SYT, Mok KW, Lee WM, Wong CKC, Cheng CY (2013) p-FAK-Tyr397 regulates spermatid adhesion in the rat testis via its effects on F-actin organization at the ectoplasmic specialization **Am J Physiol Endocrinol & Metabol** 305: E687-E699.

2. Xiao X, Mruk DD, Tang EI, Wong CKC, Lee WM, John CM, Turek PJ, Silvestrini B, Cheng CY (2014) Environmental toxicants perturb human Sertoli cell adhesive function via changes in F-actin organization mediated by actin regulatory proteins. **Human Reproduction** 29: 1279-1291.

3. Qian X, Mruk DD, Cheng Y-H, Tang EI, Han D, Lee WM, Wong EWP, Cheng CY (2014) Actin binding proteins, spermatid transport and spermiation. **Seminars in Cell & Developmental Biology** 30: 75-85.

4. Zhu W, Liu P, Chen Q, Liu Z, Yan K, Lee WM, Cheng CY, Han D (2014) p204-Initiated innate antiviral response in mouse Leydig cells. **Biology of Reproduction** 91: 1-9.

5. Xiao X, Mruk DD, Wong EWP, Lee WM, Han D, Wong CKC, Cheng CY (2014) Differential effects of c-Src and c-Yes on the endocytic vesicle-mediated trafficking events at the Sertoli cell blood-testis barrier: an *in vitro* study. **Am J Physiol Endocrinol Metab** 307: E553-E562.

6. Gao Y, Lee WM, Cheng CY (2014) Mini review: Thyroid hormone function in the rat testis. **Frontiers in Endocrinology** Vol 5 Article 188 pp1-7.

7. Tang EI, Mok KW, Lee WM, Cheng CY (2015) EB1 regulates tubulin and actin cytoskeletal networks at the Sertoli cell blood-testis barrier in male rats: an *in vitro* study. **Endocrinology** 156: 680-693.

8. Tang EI, Mruk DD, Lee WM, Cheng CY (2015) Cell-cell interaction, cell polarity and the blood-testis barrier. In *Cell Polarity 1*, K.Ebnet (ed), Springer Publishing, Chapter 13.

No.	Year of publication	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)*	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)*
9	2015	**Li N**, Mruk DD, Wong CKC, **Lee WM, Han D**, Cheng CY*	Actin bundling protein plastin 3 is a regulator of ectoplasmic specialization (ES) dynamics during spermatogenesis in the rat testis. **FASEB J** 29: 3788-3805.	Yes	Yes	Yes
10	2015	**Mok KW, Chen H, Lee WM,** Cheng CY*	rpS6, the downstream molecule of mTORC1, regulates blood-testis barrier dynamics through Arp3-mediated actin microfilament organization in rat Sertoli cells - an *in vitro* study**.** **Endocrinology** 156: 1900-1913.	Yes	Yes	Yes
11	2015	**Li N**, Mruk DD, Wong CKC, **Han D, Lee WM,** Cheng CY*	Formin 1 regulates ectoplasmic specialization in the rat testis through its actin nucleation and bundling activity. **Endocrinology** 156: 2969-2983.	Yes	Yes	Yes
12	2016	**Li N**, Mruk DD, **Mok KW**, Li MWM, Wong CKC, **Lee WM, Han D,** Silvestrini B, Cheng CY*	Connexin 43 reboots meiosis and reseals blood-testis barrier (BTB) following toxicant mediated aspermatogenesis and barrier function. **FASEB J** 30:1436-1452.	Yes	Yes	Yes
13	2016	**Li N**, Mruk DD, **Lee WM,** Wong CKC, Cheng CY	Is toxicant-induced Sertoli cell injury in vitro a useful model to study molecular mechanisms in spermatogenesis? **Seminars in Cell and Developmental Biology** 59:141-156.	Yes	Yes	Yes
14	2016	**Gao Y**, Lui WY, **Lee WM,** Cheng CY*	Polarity protein Crumbs homolog-3 (CRB3) regulates ectoplasmic specialization dynamics through its action on F-actin organization in Sertoli cells. **Scientific Reports** 6 Article Number: 28589.	Yes	Yes	Yes
15	2016	**Li** N, Mruk DD, **Chen HQ,** Wong CKC, **Lee WM,** Cheng CY*	Rescue of perfluorooctanesulfonate (PFOS)-induced Sertoli cell injury through overexpression of gap junction protein connexin 43. **Scientific Reports** 6 Article Number: 29667.	Yes	Yes	Yes
16	2016	**Tang EI, Lee WM**, Cheng CY*	Coordination of actin- and microtubule-based cytoskeletons supports transport of spermatids and residual bodies/phagosomes during spermatogenesis in the rat testis. **Endocrinology** 157: 1644-1659.	Yes	Yes	Yes
17	2016	**Chen HQ**, Mruk DD, **Lee WM,** Cheng CY*	Planar cell polarity (PCP) protein Vangl2 regulates ectoplasmic specialization dynamics via its effects on actin microfilaments in the testes of male rats. **Endocrinology** 157: 2140-2159.	Yes	Yes	Yes
18	2016	**Li N**, Mruk DD, **Tang EI, Lee WM,** Wong CKC, Cheng CY*	Formin 1 regulates microtubule and F-actin organization to support spermatid transport during spermatogenesis in the rat testis. **Endocrinology** 157: 2894-2908.	Yes	Yes	Yes

19	2016	**Gao Y**, **Xiao X**, Lui WY, **Lee WM**, Mruk DD, Cheng CY*	Cell polarity proteins and spermatogenesis. **Seminars in Cell and Developmental Biology** 59: 62-70.	Yes	Yes	Yes
20	2016	**Gao Y**, Mruk DD, Lui WY, **Lee WM**, and Cheng CY*	F5-peptide induces aspermatogenesis by disrupting organization of actin- and microtubule-based cytoskeletons in the testis. **Oncotarget J**. 7: 64203-64220.	Yes	Yes	Yes
21	2017	**Gao Y**, Mruk DD, **Chen HQ**, Lui WY, **Lee WM**, and Cheng CY*	Regulation of blood-testis barrier by a local axis in the testis: role of laminin α2 in the basement membrane" **FASEB J** 31: 584-597.	Yes	Yes	Yes
22	2017	**Gao Y**, **Chen HQ**, Lui WY, **Lee WM**, Cheng CY*	Basement Membrane Laminin α2 regulates BTB Dynamics via its effects on F-actin and microtubule (MT) cytoskeleton via mTORC1 signaling. **Endocrinology** 158: 963-978.	Yes	Yes	Yes
23	2017	**Gao Y, Chen HQ, Xiao X,** Lui WY, **Lee WM,** Mruk DD, and Cheng CY*	Perfluorooctanesulfonate (PFOS)-induced Sertoli cell injury through a disruption of F-actin organization is mediated by Akt1/2" **Scientific Reports** 7 Article Number: 1110.	Yes	Yes	Yes
24	2017	**Chen HQ**, Mruk DD, **Lee WM**, Cheng CY*	Regulation of spermatogenesis by a local functional axis in the testis: role of the basement membrane-derived noncollagenous 1 domain peptide. **FASEB J** 31:3587-3607.	Yes	Yes	Yes
25	2017	**Chen HQ**, Mruk DD, Lui WY, Wong CKC, **Lee WM**, Cheng CY*	Cell polarity and planer cell polarity in spermatogenesis. **Seminars in Cell and Developmental Biology** in press.	Yes	Yes	Yes
26	2018	**Chen HQ, Gao Y**, Mruk DD **Lee WM,** Cheng CY*	Rescue of PFOS-induced human Sertoli cell injury by overexpressing a p-FAK-Y407E phosphomimetic mutant **Scientific Reports** in press.	Yes	Yes	Yes
27	2018	**Wen Q, Tang EI, Gao Y**, Jesus T, Chu D, **Lee WM**, Wong CK, Liu YX, Xiao X, Silvestrini B, Cheng CY*	Signaling pathways regulating blood-tissue barriers - lesson from the testis. **Biochimica et Biophysica Acta-- Biomembrane** 1860: 141-153.	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)*

August 3-7, 2015 Vienna, Austria	Planar cell polarity protein Van Gogh-like 2 (Vangl2) regulates Sertoli cell blood-testis barrier (BTB) function and actin cytoskeleton dynamics	14th International Congress on Amino Acids and Proteins	No	Yes	Yes	Yes
July 3-6 2016 Helsinki, Finland	Gap junction Connexin 43 regenerates meiosis and toxicant-induced blood-testis barrier disruption in the rat testes	32nd Congress of the European Society for Human Reproduction and Embryology	No	Yes	Yes	Yes
July 2-5 2017 Geneva, Switzerland	The downstream signaling pathways of perfluorooctanesulfonate (PFOS)-induced disruption of blood-testis barrier -- disorganization of F-actin-based and microtubule-based cytoskeleton in Sertoli cells.	33rd Congress of the European Society for Human Reproduction and Embryology	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
Miss EI Tang	PhD	September 2012	July 2016/Oct 2016
Mr HQ Chen	PhD	September 2013	June 2017/Oct 2017

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

Collaborations with Dr. D Han at Bejing Union Medical College, Beijing and Dr. CY Cheng at Population Council, New York. Training of postdoctoral fellows Drs Li Nan, Gao Ying and Wen Qing who were first and coauthors of the research output publications.