Biochimica et Biophysica Acta 1860 (2018) 141–153

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Signaling pathways regulating blood–tissue barriers — Lesson from the testis☆

Qing Wen [a], Elizabeth I. Tang [a], Ying Gao [a], Tito T. Jesus [a], Darren S. Chu [a], Will M. Lee [b], Chris K.C. Wong [c], Yi-Xun Liu [d], Xiang Xiao [e], Bruno Silvestrini [f], C. Yan Cheng [a,*]

[a] The Mary M. Woldford Laboratory for Male Contraceptive Research, Center for Biomedical Research, Population Council, 1230 York Ave, New York, NY 10065, United States
[b] School of Biological Sciences, University of Hong Kong, Hong Kong, China
[c] Department of Biology, Hong Kong Baptist University, Hong Kong, China
[d] State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China
[e] Department of Reproductive Physiology, Zhejiang Academy of Medical Sciences, Hangzhou 310013, Zhejiang, China
[f] S.B.M. Pharmaceuticals Srl, Rome, Italy

ARTICLE INFO

Article history:
Received 23 January 2017
Received in revised form 18 April 2017
Accepted 21 April 2017
Available online 25 April 2017

Keywords:
Testis
Spermatogenesis
Blood–testis barrier
Blood–tissue barriers
Seminiferous epithelial cycle
Sertoli cell
Germ cell
mTOR
mTORC1
mTORC2
Microtubules
F-actin
Cytoskeleton
Motor proteins
Nucleation proteins

ABSTRACT

Signaling pathways that regulate blood–tissue barriers are important for studying the biology of various blood–tissue barriers. 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. This article is part of a Special Issue entitled: Gap Junction Proteins edited by Jean Claude Herve.

Contents

☆ This article is part of a Special Issue entitled: Gap Junction Proteins edited by Jean Claude Herve.

* Corresponding author.
E-mail addresses: Y-Cheng@popcbr.rockefeller.edu, ccheng@rockefeller.edu (C.Y. Cheng).

http://dx.doi.org/10.1016/j.bbamem.2017.04.020
0005-2736/

1. Introduction

One of the major functions of blood–tissue barriers is to seal vital organs such as the brain, eye, intestine and testis from external cues and harmful substances in the environment. These barriers safeguard key physiological processes in tissue behind the tight junction (TJ)-barrier of the corresponding organ through restrictive paracellular and/or transcellular diffusion. Examples of such barriers are the blood–brain barrier (BBB), the blood–testis barrier (BTB), the blood–retinal barrier, the gut barrier and others [1–7]. However, many of these tissue barriers are not static ultrastructures. Instead, tight junctions (TJs) at the barriers undergo dynamic changes, requiring continuous disassembly, reassembly and stabilization/maintenance. In this way, for example, nutrients can be absorbed in the gut (or intestine) [8,9], and preleptotene spermatocytes in the testis, connected in clones via intercellular bridges [10,11], can be transported across the BTB at stage VIII of the epithelial cycle of spermatogenesis [12–15], without compromising the integrity of the corresponding blood–tissue barrier. Thus, it is obvious that this timely remodeling of blood–tissue barriers is under tight physiological control, involving signaling pathways which facilitate the remodeling (i.e., opening, closing, and stabilization) of the barrier. This also prevents unwanted exposure of an organ or tissue to pathogens and/or harmful substances. Studies in the past decade have made important advances in understanding the signaling pathways that play crucial roles in blood–tissue barrier dynamics [7,16]. Herein, studies based on the BTB in the testis have illustrated the involvement of several signaling complexes and pathways that are intimately involved in regulating BTB dynamics during the epithelial cycle of spermatogenesis. More important, based on these findings, it is becoming increasingly clear that these signaling pathways exert their regulatory effects downstream by targeting the F-actin- and/or microtubule (MT)-based cytoskeletons of the Sertoli cell in the seminiferous epithelium, wherein specialized junctions between adjacent Sertoli cells create the BTB. It is conceivable that these findings and concepts are applicable to other blood–tissue barriers, at least in tissue barriers that may utilize similar signaling complexes and/or pathways to modulate their function.

Understanding blood–tissue barriers garnered interest largely due to the difficulties faced by clinicians in treating diseases of the brain and the testis, since the BBB and BTB virtually block the entry of most drugs, including most chemotherapeutic drugs and male contraceptives, from exerting their effects in the target organs behind the barriers. This is possibly contributed by the presence of an extensive network of drug transporters at the BBB, and also at the BTB [3,5,17–20]. For instance, studies have shown that the testis serves as a reservoir for some viruses, mostly notably human HIV [21,22] (and Zika virus, recently shown in mice [23]). Surprisingly, although viral concentration in the serum of HIV-infected patients receiving suppressive antiretroviral therapy (ART) can be virtually undetectable, viral levels remain exceedingly high in the testis of these patients [24]. These findings thus illustrate some viruses have the ability to evade the physiological barrier conferred by the BTB or the BBB to gain entry into the testis or the brain, respectively. In fact, based on the use of human brain microvascular endothelial cells as a BBB study model, it has been demonstrated that Japanese encephalitis virus has the ability to disrupt the BBB by compromising the tight junction (TJ)-permeability barrier through changes in the host cell apoptotic protein expression [25]. Also, Hepatitis E virus has also been shown to disrupt the BBB in the brain by reducing the expression of ZO-1 at the endothelial TJ-barrier [26]. Additionally, mice transfected with Zika viruses have been shown to infect preferentially Sertoli cells, spermatogonia and primary spermatocytes in the testis [23], illustrating their ability to pass through the BTB. On the other hand, HIV-1 viruses are known to penetrate through the BBB mediated by their Tat and gp120 proteins that are capable of down-regulating the expression of adhesion proteins (e.g., occludin, claudins and ZO-1) considerably at the capillary endothelial cells, making the barrier "leaky" to facilitate their passage at the barrier [27–30]. Collectively, these findings support the notion that viruses have the ability to evade the TJ-barrier in multiple blood–tissue barriers such as the BBB and the BTB to infect an organ, creating a reservoir to harbor the viruses. There are indications that this unusual level of viruses in the brain and the testis is supported by the presence of an extensive network of drug transporters at the BBB [5,17,19] and the BTB [3,18,20,31], which prevent therapeutic drugs, that are supposed to eradicate viruses, from passing through the corresponding barrier. For instance, P-glycoprotein, an active drug transporter, is capable of pumping antiretroviral drugs (e.g., atazanavir) or male contraceptive drug (e.g., adjudin) out of the brain or the testis, respectively [17,18,32,33]. Thus, studies have supported the concept that viruses (e.g., HIV and Zika) elect to use the testis and the brain as a viral sanctuary site [34–36]. In the testis, besides the presence of drug transporters, this is likely due to the immune tolerance properties conferred by the Sertoli cells, possibly through the immunosuppressing biomolecules secreted by these cells, making the testis an immune privileged site [37–43] in particular at the adluminal (apical) compartment behind the BTB to accommodate post-meiotic spermatid development (Fig. 1). These immunosuppressive biomolecules, coupled with the presence of an extensive network of antiretroviral drug transporters and drug metabolic enzymes in the testes of both rodents and humans [18,44,45], thus prevent the entry of anti-viral drugs into the testis, making the testis a safe haven for some viruses [34,35]. In light of these findings, a better understanding on the biology of blood–tissue barriers will have significant clinical implications in treating (or preventing) various diseases. In this brief review, we thought it pertinent to focus findings based on studies of the BTB which shall provide helpful information in designing functional studies of other blood–tissue barriers.

2. Blood–testis barrier *versus* other blood–tissue barriers

Most tissue barriers such as the BBB and the blood–retinal barrier are constituted by TJ-barrier of adjacent endothelial cells of microvessels supported by pericytes, surrounding the organ/tissue behind the barrier, to regulate transcellular and paracellular transports of substances across the barrier. For instance, the BBB is conferred by endothelial TJ-barrier and pericytes, and supported by astrocytes and neurons in the brain [1,46–48] (Fig. 1). It is of interest to note that the BTB, in contrast, is conferred by TJs between adjacent Sertoli cells and is restrictively located to the basal region of the seminiferous epithelium, adjacent to the basement membrane [3,4,49–53] since microvessels located in the interstitial space between the seminiferous tubules contribute virtually no barrier function to the Sertoli cell BTB [3,50,54]. This is somewhat similar to the blood–intestine (or the gut) barrier and the blood–epididymis barrier which are created by the epithelial cells of the small intestine and the epididymis [55,56]. However, besides the TJs, the BTB is supported by a testis-specific actin-rich atypical adherens junction called ectoplasmic specialization (ES) [49,57–59]. Since the BTB is anatomically localized at the basal region of the seminiferous epithelium,

towards the base of the seminiferous tubule, the ES found near the basement membrane is designated basal ES (Fig. 1). However, for other blood–tissue barriers, such as the blood–retina barrier, the TJ-barrier, in contrast to the BTB, is located the furthest away from the basement membrane, whereas the BTB is closest to the basement membrane (Fig. 1) [60]. Interestingly, the basal ES coexists with TJ, and they are further supported by gap junction (GJ), which together with the intermediate filament based desmosome all constitute the BTB, making it one of the tightest and also dynamic blood–tissue barriers in the mammalian body [3,49,52,61] (Fig. 1). Using electron microscopy, the basal ES is composed of two arrays of actin microfilament bundles that are sandwiched in between the cisternae of endoplasmic reticulum (ER) and the apposing Sertoli cell plasma membrane (Fig. 1) [3,49,62–64]. These actin microfilament bundles thus confer the BTB its unusual adhesive function.

The BTB is also supported by two prominent signaling proteins derived from mTOR (mammalian target of rapamycin) known as mTORC1 (mammalian target of rapamycin complex 1) and mTORC2 (mammalian target of rapamycin complex 2) [65,66], as well as FAK (focal adhesion kinase) in particular p-FAK-Tyr407 [3,67]. Recent studies have shown that these signaling protein complexes also exert their regulatory effects through the F-actin and/or microtubule (MT)-based cytoskeletons [68–72], consistent with emerging evidence in the field in which mTOR was also shown to exert its regulatory effects through the cytoskeleton [73]. Anatomically, the BTB divides the seminiferous epithelium into the basal and the adluminal compartments (Fig. 1), so that meiosis I/II and post-meiotic spermatid development all take place behind the BTB, whereas undifferentiated spermatogonia (including spermatogonial stem cells), differentiated type A and type B spermatogonia, and also the newly transformed preleptotene spermatocytes from type B spermatogonia reside outside the BTB. Once formed at stage VIII of the epithelial cycle in the rodent testis, preleptotene spermatocytes are the only germ cells that are actively transported across the immunological barrier at stage VIII of the epithelial cycle in both rat and mouse testes *vs.* stage III in human testes [12–14,74–76]. In this context, it is of interest to note that the BTB is a highly dynamic ultrastructure since preleptotene spermatocytes connected in clones via intercellular bridges [10,11] differentiated from type B spermatogonia must be transported across the BTB between Sertoli cells at stage VIII of the epithelial cycle over the course of 20.8 and 29.1 h in mouse and rat testes [12,13] *vs.* 24 h at stage III in human testes [74]. Thus, the paracellular transport of preleptotene spermatocytes takes place rather rapidly, illustrating this process must be tightly coordinated and regulated, likely through the involvement of multiple signaling pathways. Interestingly, the BTB is not compromised (i.e., becoming "leaky") during the transport of preleptotene spermatocytes across the barrier, even transiently, in order to avoid unwanted entry of harmful substances from systemic circulation to the adluminal compartment to perturb post-meiotic spermatid development. At the same time, specific antigens residing in late spermatocytes and post-meiotic spermatids behind the BTB in the adluminal compartment are sequestered from the host immune system. Taken collectively, these earlier findings have shown that the BTB has some distinctive morphological features, and its dynamic remodeling that accommodates the transport of preleptotene spermatocytes during the epithelial cycle is a cytoskeletal-dependent cellular event. Thus, its biology is likely similar to the transport of nutrients or biomolecules across the intestinal barrier during food absorption or the BBB via transcellular or paracellular pathways. It is obvious that the precise regulatory mechanism(s) underlying these events regarding transcellular and paracellular (and active) transports in different blood–tissue barriers must be carefully evaluated in future studies. In this context, it is of interest to note that ES is also found in the apical compartment where it is designated apical ES and is restrictively expressed at the Sertoli-spermatid (steps 8–19 in the rat or 8–16 in the mouse testis) interface [77,78]. Morphologically, the apical and the basal ES share virtually similar features except that there is only a single array of actin filament bundles found in the Sertoli cell adjacent to the apposing Sertoli-spermatid plasma membranes at the apical ES *vs.* two arrays of actin filament bundles for basal ES (Fig. 1). However, the constituent proteins are somewhat different between the two. For instance, nectin 3 [79], JAM-C [80], α6β1-integrin [81–84] and laminin-α3/laminin-β3/laminin-γ3 chains [85–88] are restrictively expressed at the apical ES, whereas nectin 2 [79], connexin 43 (Cx43) [89–93] are found in both the apical and basal ES. Additionally, some proteins are mostly found at the basal ES, such as N-cadherin [94–96] with its presence at the apical ES limited to only stage I–VI tubules [97].

3. mTOR: mTORC1 *vs.* mTORC2 and BTB dynamics

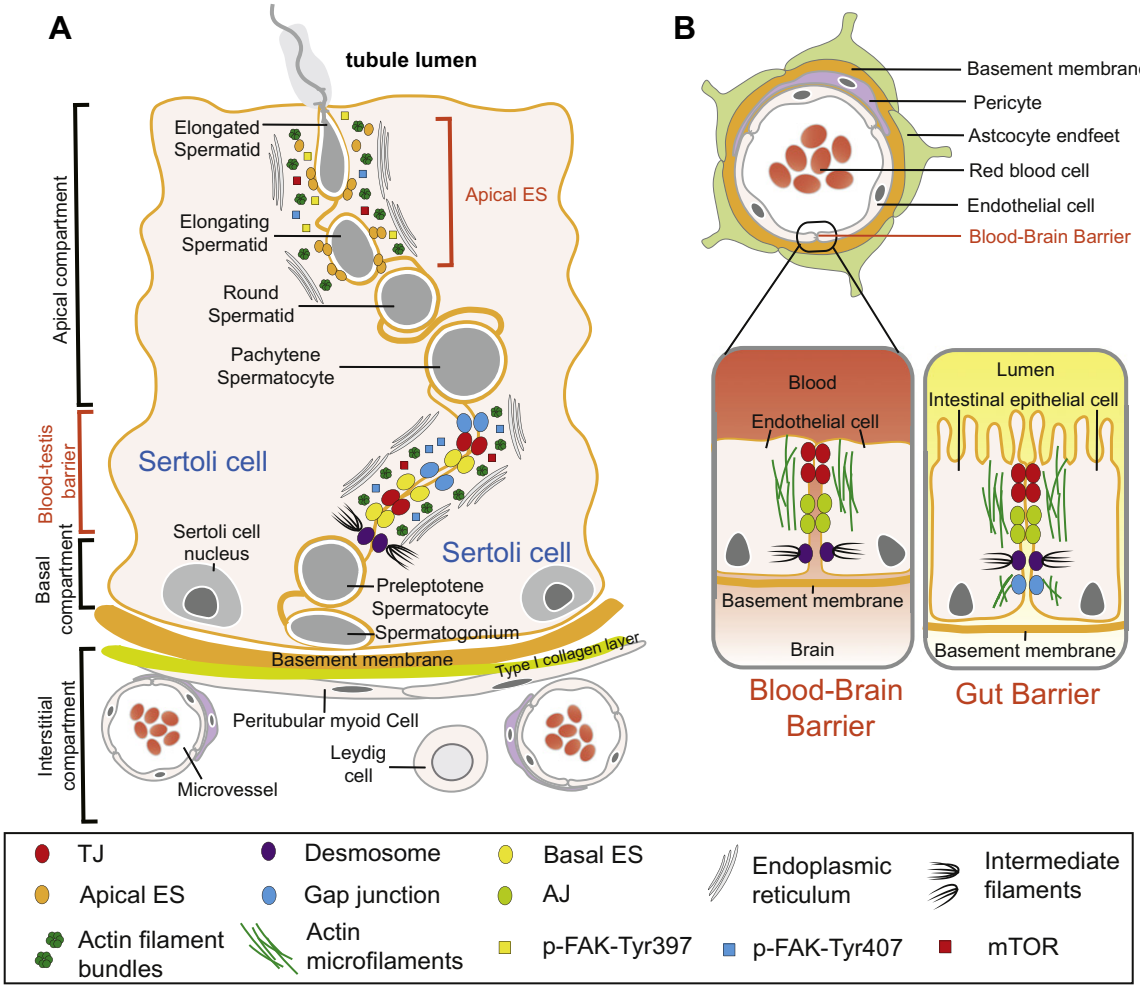
3.1. Introduction

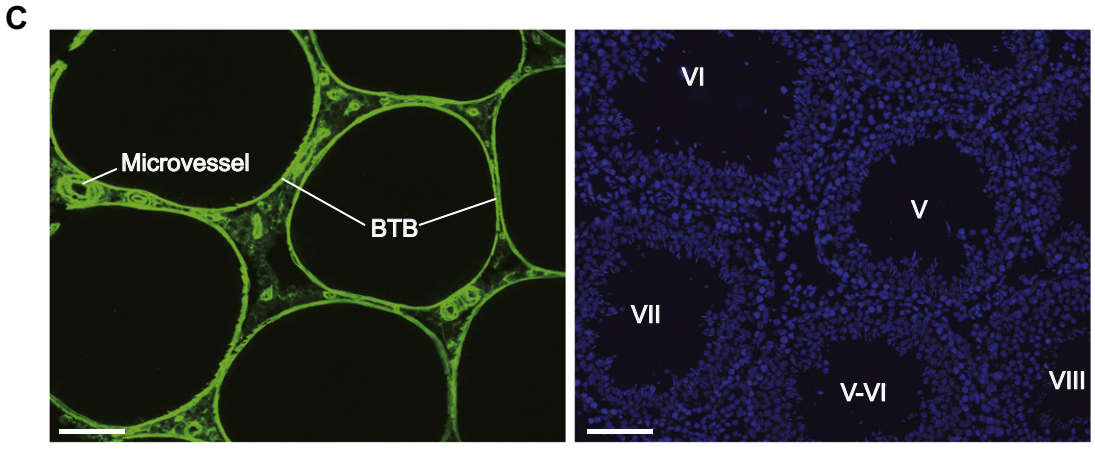
mTOR is a 290 kDa Ser/Thr protein kinase first found in yeasts, and its intrinsic kinase activity can be rapidly blocked by antibiotic rapamycin (also known as sirolimus) [98,99]. Subsequent studies have shown that mTOR is an almost indispensable signaling protein and it is found in virtually all mammalian cells, playing an important role in regulating cell energy status, growth, pathogenesis of many diseases including diabetes and carcinogenesis, and also cytoskeletal function and organization [73,98,100–103]. It is now known that mTOR exists in two distinct and functionally different forms depending on its binding partners, creating two separate signaling complexes called mTORC1 and mTORC2 [73,98,99,104]. In short, when mTOR associates with adaptor protein Raptor (regulatory-associated protein of mTOR), the mTOR/Raptor complex creates the mTORC1 signaling complex which is a rapamycin sensitive regulatory protein kinase. When mTOR associates with adaptor protein Rictor (rapamycin-insensitive companion of mTOR), the mTOR/Rictor complex creates mTORC2 which is rapamycin insensitive. The most important downstream signaling proteins of mTORC1 are p70S6K (ribosomal protein S6 kinase), rpS6 (ribosomal protein S6) and Akt1/2 *vs.* PKC-α (protein kinase C-α) and SGK1 (serum/glucocorticoid regulated kinase 1, a Ser/Thr protein kinase) signaling proteins for mTORC2 [66,103,105]. Studies have shown that treatment of spermatogonia in vitro with rapamycin, which blocks mTORC1 function through the mTORC1/p70S6K pathway, is known to reduce spermatogonial mitotic proliferation, illustrating mTORC1 is involved in the maintenance of germline pool in the rodent testis [106]. Inhibition of mTORC1 by rapamycin in vivo leads to atrophy of seminiferous tubules, associated with reduced sperm count, confirming the notion that mTORC1 is involved in spermatogenesis [106]. In this context, it is of interest to note a conditional knockout (KO) mouse model to eliminate *Raptor* was prepared by deleting mTORC1 in the Wolffian duct during development [107]. These $Raptor^{fl/fl}$;*Ksp-Cre* mice are infertile, displaying defects in the epithelial cells of their epididymis, vas deferens, and seminal vesicles due to extensive organ regression with advancing age [107]. For instance, the epididymal epithelial cells of these $Raptor^{flox/flox}$;*Ksp-Cre* mice have defects in metabolism and a considerable reduction in protein secretion [107]. Furthermore, these epithelial cells are also found to actively engulf and destroy epididymal spermatozoa in the epididymis which are not detected in the corresponding age-matched control ($Raptor^{fl/fl}$) mice [107]. In short, these findings thus illustrate the significance of mTOR in testis function but also other accessory male reproductive organs such as the epididymis, vas deferens and the seminal vesicles.

3.2. mTOR and stage-specific expression/localization of its component proteins in the testis

Studies in the testis by immunohistochemistry and/or immunofluorescence microscopy have shown that mTOR [70], and the signaling proteins of mTORC1 such as p-rpS6 (the activated form of rpS6) [69,72], and p-Akt1 and p-Akt2 (the signaling partners of rpS6) [71], and also Rictor (the adaptor/partner protein that creates mTORC2 when

binding to mTOR) [70], are all localized stage-specifically at the basal ES/BTB, and the apical ES [69–72] during the epithelial cycle. For instance, mTOR is found at the basal ES in virtually all stages of the epithelial cycle in the rat testis, but also at the apical ES at stages I–VIII of the cycle until late VIII when spermiation takes place and its staining re-appears at the apical ES in stages XIII–XIV [70]. For p-rpS6, it is predominantly expressed at the basal ES/BTB in stage VIII and IX tubules and virtually undetectable in other staged tubules; its expression at the apical ES is most predominant in stage VII tubules but considerably diminished in stage VIII and other staged tubules [71,72]. For Rictor, its expression at the basal ES/BTB is predominant at stage V–VII tubules but virtually undetectable at VIII and other stages [70]. These findings regarding their differential expression at the basal ES/BTB thus suggest that the mTORC1/rpS6 and mTORC2/Rictor signaling complexes may have different effects on the Sertoli cell BTB function. Indeed, studies in vitro and/or in vivo using different research tools have demonstrated mTORC1 and mTORC2 have antagonistic effects on the Sertoli cell BTB function. Such tools include overexpression of a constitutively active quadruple mutant of rpS6, namely a p-rpS6 mutant, which was generated by converting S235, S236, S240 and S244 (S, Ser) to E235, E236, E240 and E244 (E, Glu) [71];the use of rpS6-specific shRNA to silence rpS6 to impede mTORC1 function [69]; and the use of Rictor-specific siRNA





duplexes to silence Rictor to impede mTORC2 function [70]. In brief, mTORC1 is now known to promote Sertoli cell BTB remodeling by making the BTB "leaky" based on studies *in vitro* and also *in vivo* [69,71,72], whereas mTORC2 promotes BTB integrity by "tightening" the Sertoli cell TJ-barrier [70] to confer integrity of the BTB. These findings are important, since the presence of these two signaling complexes that have antagonistic effects at the basal ES/BTB, their differential spatiotemporal expression at the microenvironment of the BTB thus fine-tunes the timely restructuring of the Sertoli cell-cell junction to accommodate the transport of preleptotene spermatocytes across the barrier (Fig. 2). Below is a brief critical review of these findings.

3.3. mTORC1 and BTB dynamics

In mammalian cells and tissues, mTORC1 is activated by growth factors (e.g., insulin, IGF-1), nutrients (e.g., amino acids) or cellular energy status (e.g., high ATP/AMP ratio), involving PI3-K (phosphoinositide 3-kinase) and Akt/PKB upstream [66,108]. This in turn activates ribosomal protein S6K (S6 protein kinase, also known as p70 S6K) and rpS6 (ribosomal protein S6) downstream via phosphorylation to modulate anabolic metabolism and other cellular function. It is now known that Hippo, Notch, and Wnt signaling pathways also contribute to modulating mTORC1 signaling function [104]. Studies in the testis have shown that mTORC1/rpS6 signaling complex regulates BTB dynamics by promoting BTB remodeling, making the BTB "leaky" [65,66]. This conclusion is reached based on the following observation. First, a knockdown of rpS6 by RNAi (by effectively blocking mTORC1 function) promotes the Sertoli cell TJ-barrier by making it "tighter" *in vitro* or *in vivo* using either rpS6-specific siRNA or shRNA for transfection in Sertoli cells cultured in vitro or by administering into the testis *in vivo*, respectively [69]. Second, treatment of Sertoli cells with an established TJ-permeability barrier that mimics the BTB *in vivo* with rapamycin (mTORC1 is rapamycin sensitive) that blocks mTORC1 function also causes the Sertoli cell TJ-barrier to become "tighter" *in vitro* [69]. Third, overexpression of an rpS6 cDNA (wild-type) in Sertoli cells (i.e., by activating/promoting mTORC1 function) perturbs the Sertoli cell TJ-barrier, making it "leaky" [71,72]. However, overexpression of p-rpS6 mutant (the constitutively activated form of rpS6) in Sertoli cells causes the Sertoli TJ-barrier to be "leakier" than overexpression of wild-type rpS6 [71,72]. More important, mTORC1/rpS6 complex is known to exert its disruptive effects on the Sertoli cell BTB integrity by perturbing F-actin organization at the apical and basal ES [69], through a considerable reduction in actin bundling activity, thereby perturbing actin bundle organization at the ES [72]. In short, these findings support the notion that mTORC1/rpS6 signaling complex promotes BTB remodeling by disrupting F-actin organization at the basal ES, causing the BTB to become "leaky", which is what occurs at stage VIII of the epithelial cycle. Additionally, these findings are supported by the stage-specific expression of p-rpS6 at the basal ES/BTB wherein p-rpS6 S235/S236 and p-rpS6 S240/S244 expression was found to be up-regulated considerably at late stages VIII–IX [69,71,72] during BTB remodeling to facilitate the transport of preleptotene spermatocytes at the immunology barrier.

It is increasingly clear that mTORC1 modulates BTB dynamics through two signaling pathways (Fig. 2). First, an activation of rpS6 through an up-regulation of p-rpS6 (i.e., an induction of mTORC1 signaling function) leads to a down-regulation of p-Akt1/2, which in turn induces an increase in the association of Arp3 and its upstream activator N-WASP (neuronal Wiskott-Aldrich syndrome protein) [72]. This thus causes actin nucleation at the barbed ends of a linear actin microfilament (i.e., branched actin polymerization), re-organizing actin filaments from a bundled to a branched/un-bundled network, destabilizing cell adhesion protein complexes at the BTB (e.g., occludin-ZO-1, N-cadherin-α-catenin which utilize F-actin for attachment) due to a loss of actin filament bundles as the basal ES/BTB. Indeed, there is considerable reduction in actin bundling activities in Sertoli cell epithelium following overexpression of the constitutively active quadruple phosphomimetic mutant p-rpS6 [72]. This latter change thus contributes to destabilizing the arrays of actin microfilament bundles at the basal ES necessary to maintain BTB integrity, making the immunological barrier "leaky". In this context, it is of interest to note that the ability of the Sertoli cell to organize the actin microfilaments between their bundled and un-bundled/branched configuration is crucial to BTB dynamics. For instance, a Sertoli cell-specific knockout (KO) of N-WASP was shown to cause male infertility in both *Drosophila* and mouse [109], and a detailed analysis has shown that this is due to the inability of the testis to confer plasticity to the F-actin network at the ES to support meiosis and spermiogenesis, leading to irreversible disruption of the basal ES/BTB to support spermatogenesis [110]. Second, overexpression of the rpS6 phosphomimetic (and constitutively active) mutant p-rpS6 in Sertoli cell epithelium that leads to a down-regulation of Akt1/2 is shown to induce MMP-9 (matrix metalloproteinase 9) production by Sertoli cells, which in turn induces proteolysis of TJ and/or basal ES adhesion protein complexes at the BTB, contributing to a "leaky" immunological barrier [71]. This mTORC1 based p-rpS6-p-Akt1/2-MMP-9 signaling pathway that modulates BTB function is confirmed by studies using MMP-9 inhibitor 1 (a specific MMP-9 inhibitor) since this MMP-9 inhibitor effectively blocks the p-rpS6 mutant-induced Sertoli cell TJ-barrier disruption [71]. Also, a knockdown of p-Akt1/2 by RNAi using

Fig. 1. A schematic diagram illustrating the layout of various junction types at the blood–testis barrier (BTB) versus other blood–tissue barriers. (A) In the testis, the Sertoli cell BTB physically divides the seminiferous epithelium inside a seminiferous tubule, such as a stage VII tubule in an adult rat testis, into the apical (adluminal) and the basal compartments as shown herein. Preleptotene spermatocyte differentiated from type B spermatogonium in late stage VII of the cycle is the only spermatocyte that is being transported across the immunological barrier at stage VIII of the cycle. The most notable adherens junction (AJ), a cell-cell actin-based anchoring junction type, is the testis-specific atypical AJ called ectoplasmic specialization (ES). The ES is either found in the adluminal compartment at the Sertoli-spermatid (step 8-19 spermatids in the rat testis) interface called apical ES or at the Sertoli cell-cell interface called the basal ES, adjacent to the basal compartment. The ES is typified by the presence of an array of actin filament bundles in the Sertoli cell that is sandwiched in between the endoplasmic reticulum and the adjacent Sertoli-spermatid plasma membranes (apical ES) or the adjacent Sertoli cell-cell plasma membranes (basal ES). Unlike other blood–tissue barriers, the BTB is closest to the basement membrane and it is constituted by co-existing TJ, gap junction and desmosome (a cell-cell intermediate filament-based anchoring junction). As noted herein, a pachytene spermatocyte differentiates further into diplotene spermatocyte so that it can undergo meiosis I/II to form round spermatids which will be further developed into sperm via spermiogenesis. In the rat and mouse testis, apical ES, once formed between Sertoli cells and step 8 spermatids, is the only anchoring device that supports spermatid adhesion. This is also the anchoring device that supports spermatid transport across the adluminal compartment so that spermatozoa line-up against the tubule lumen to prepare for their release at spermiation at late stage VIII of the epithelial cycle. The seminiferous epithelium composed of only Sertoli and germ cells at different stages of their development lying on the basement membrane which is a modified form of extracellular cell matrix [86,144]. Behind the basement membrane is the type I collagen layer, to be followed by the peritubular myoid cell layer. Microvessels and Leydig cells are found in the interstitial space. (B) Other blood–tissue barriers, such as the blood–brain barrier are constituted mostly by endothelial TJ-barrier between endothelial cells or the TJ-barrier between epithelial cells as shown in the gut barrier herein. These TJ-barriers, unlike the BTB, are furthest away from the basement membrane and AJ that form the adherens plaque always located behind the TJ, which together form the junctional complex, to be followed by desmosome and/or gap junction. In short, the different cell junction types in other blood–tissue barriers do not mix as co-existing ultrastructures as seen at the BTB. Pericytes are also an integrated component of the BBB, which is also supported by astrocytes and neurons. (C) A functional assay to illustrate the relative location of the BTB in the rodent testis and the ability of this immunological barrier to block the diffusion of biotin across the Sertoli cell TJs is shown herein. The functional BTB integrity assay in vivo was performed as detailed elsewhere [147]. Herein, a membrane impermeable biotin (EZ-Link Sulfo-NHS-LC-biotin, Mr. 556.59 obtained from Thermo Fisher Scientific) was administered to the testis under the tunica albuginea when the rat was under anesthesia using Ketamine HCl/xylazine as described [147]. About 30 min thereafter, rats were euthanized by CO_2 asphyxiation, frozen sections of the testis were obtained and biotin was detected by Alexa Fluor 488-streptavidin (green fluorescence). In this normal rat testis as shown in the *left panel*, the BTB residing near the basement membrane, as annotated, blocked the diffusion of the biotin (see green fluorescence) across the barrier in all tubules as noted herein. However, the microvessels found in the interstitial space were not effective in blocking the diffusion of biotin to the seminiferous tubules. *Right panel* is the corresponding DAPI (4′,6-diamidino-2-phenylindole) image (stained for the cell nuclei) of the *left panel* illustrating the different staged tubules. Scale bar, 100 μm.

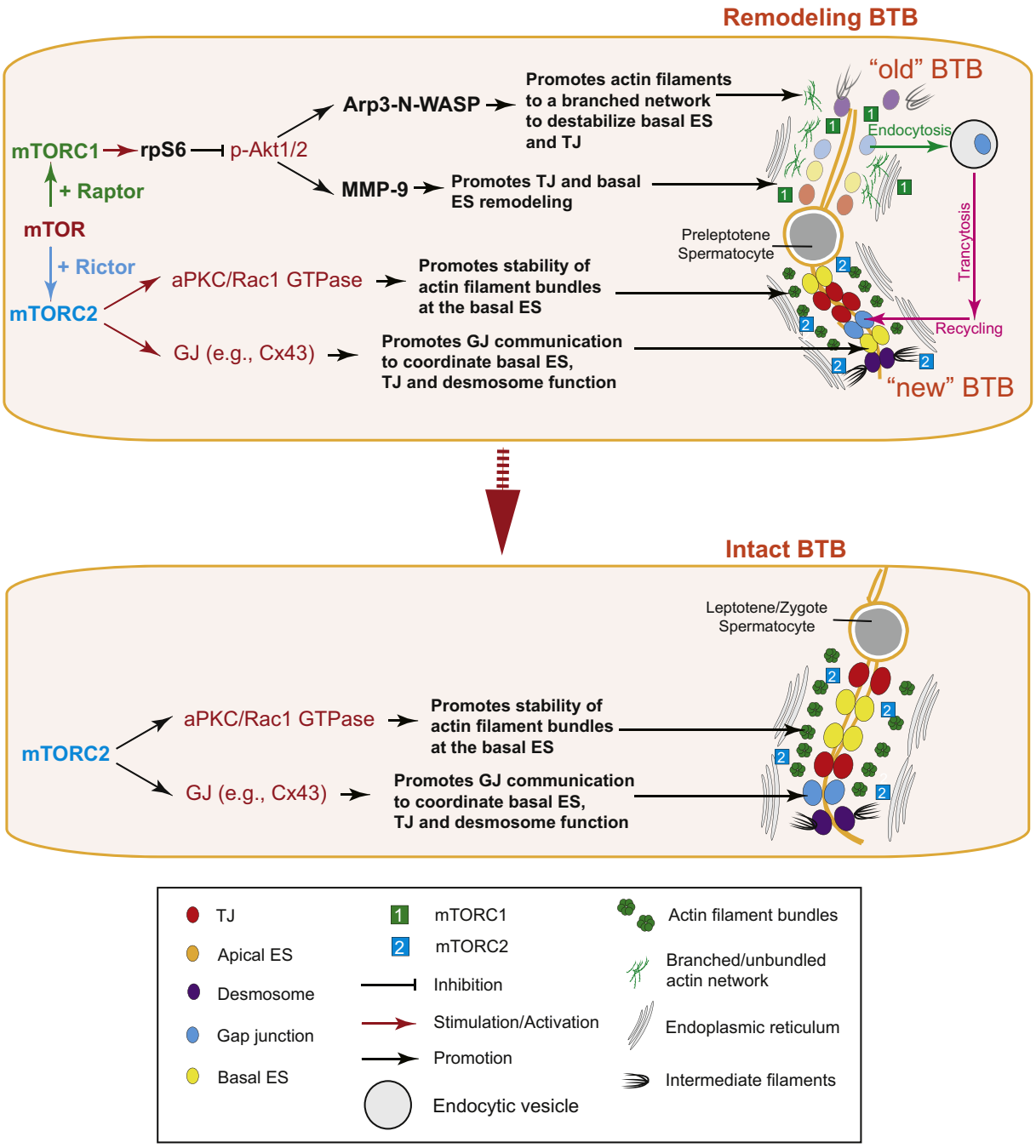


Fig. 2. A schematic diagram illustrating the involvement of mTORC1 and mTORC2 in BTB dynamics. mTORC1 and mTORC2 are known to promote BTB remodeling and BTB integrity, respectively. It is through the antagonistic effects of these two mTOR signaling proteins, mTORC1 and mTORC2 can rapidly modulate the opening and/or closing of the BTB across the microdomains of the BTB during the transport of preleptotene spermatocytes connected in clones across the immunological barrier. mTORC1 (a complex formed by mTOR and Raptor) exerts its effects through rpS6 and the p-Akt1/2 signaling molecule involving both the Arp2/3 complex and its upstream activator N-WASP, and MMP9 which affect the actin organization and the relative stability of adhesion protein complexes at the BTB. mTORC2 (a complex formed by mTOR and Rictor) exerts its effects through aPKC (atypical protein kinase C)/Rac1 GTPase, and gap junction (GJ, such as Cx43-based GJ communication channels). See text for further details.

specific siRNA duplexes also mimics findings of p-rpS6 mutant overexpression in Sertoli cells by perturbing the Sertoli cell TJ-permeability barrier function [71]. More important, a knockdown of p-Akt1/2 by RNAi in Sertoli cells also leads to an increase in Arp3-N-WASP association, analogous to overexpression of p-rpS6 mutant in Sertoli cells [72]. Taken collectively, these findings provide compelling evidence that mTORC1/rpS6 signaling complex exerts its disruptive effects on the Sertoli TJ-permeability barrier through p-rpS6/Akt1/2/Arp3-N-WASP or p-rpS6/Akt1/2/MMP-9 signaling pathways. Nonetheless, some of these findings remain to be confirmed and expanded to studies in the adult rat testes in vivo to assess if an overexpression of the p-rpS6 phosphomimetic mutant (or an inactivation of the Akt1/2 signaling protein) would impede BTB function as seen in the studies *in vitro*.

3.4. mTORC2 and BTB dynamics — an involvement of GJ function?

Studies have shown that mTORC2 promotes BTB integrity, keeping the BTB "tight" in the testis [65,66], based on the following observations. First, a knockdown of Rictor (Rictor binds to mTOR to form the mTORC2 complex, and thus its knockdown effectively blocks mTORC2 function)

by RNAi perturbs the Sertoli cell TJ-barrier *in vitro*, making it "leaky" [70]. Second, Rictor knockdown in the testis *in vivo* by RNAi also makes the BTB "leaky", mediated through disruptive organization of F-actin at the ES in the testis [70]. Furthermore, Rictor knockdown by RNAi in Sertoli cells with an established TJ-permeability barrier also perturbs the interaction of the basal ES adaptor protein α-catenin with actin, as well as TJ adaptor protein ZO-1 with actin [70]. These observations are physiologically important since it is known that α-catenin and ZO-1 anchor the corresponding basal ES integral membrane protein N-cadherin and TJ integral membrane protein occludin (also JAM-A and claudin 11) to F-actin, respectively, for attachment. A loss of Rictor (i.e., mTORC2 function) by RNAi would lead to changes in N-cadherin-α-catenin and occludin-ZO-1 association, this thus illustrates that mTOR/Rictor (i.e., mTORC2) plays a crucial role in modulating protein–protein interactions of cell adhesion protein complexes at the BTB. Furthermore, specific inactivation of mTORC2 in Sertoli cells using a genetic model by deleting *Rictor* in Sertoli cells in *Rictor*$^{flox/flox}$;*Amh-Cre* mice, led to infertile mice with defects in actin and MT organization in Sertoli cells across the seminiferous epithelium. Loss of cytoskeletal organization thereby impeded Sertoli cell polarity and BTB function, resulting in spermatogenic arrest, in which the tubules of these adult mice were devoid of elongating/elongated spermatids [111]. Findings from the genetic model thus confirm the notion that mTORC2 is supporting Sertoli cell function including BTB integrity through its effects to promote cytoskeletal organization in Sertoli cells. Besides these observations, it was shown that a Rictor knockdown also impeded Sertoli cell-cell GJ-based channel communication based on FRAP (fluorescence recovery after photobleaching) analysis [70], suggesting the possibility that mTORC2 may exert its regulatory effect through GJs.

While the testis is known to express almost a dozen GJ proteins during the epithelial cycle including the testis-specific connexin 33 [112,113], connexin 43 (Cx43) is one of the most important and the best studied GJ proteins in the testis in both rodents and humans [89–91,114,115]. In fact, a specific knockout (KO) of Cx43 leads to male infertility [92,116,117]. In these Sertoli specific-Cx43 KO mice, spermatogonia fail to differentiate into spermatocytes to initiate meiosis I/II, and Sertoli cells also fail to become terminally differentiated to support the establishment of a functional BTB [116]. Since Sertoli cells remain proliferative, the Cx43-specific KO in Sertoli cells leads to a surge in Sertoli cell population, and clusters of Sertoli cells are persistently found in tubule lumen in adult mouse testes, failing to support spermatogenesis [116,118]. These findings also suggest that other connexins found in the testis fail to supersede the loss function of Cx43 following its specific deletion in Sertoli cells. However, in these earlier studies [92,116,117], the expression of other connexins found in the testis (e.g., Cx33) has not been examined so that it is not known if other connexin genes were affected following Sertoli cell-specific deletion of Cx43. Nonetheless, subsequent studies have shown that germ cell-specific deletion of Cx43 fail to induce any phenotypic changes in the seminiferous epithelium of these mouse testes since Sertoli cells in these mice express Cx43, displaying normal spermatogenesis and fertility even though the steady-state level of Cx43 in these mutant mouse testes is considerably reduced since contribution of Cx43 to the testis pool by germ cells has been voided [119]. Collectively, these findings thus support the notion that Cx43-based GJ communications between Sertoli cells (perhaps also at the Sertoli–germ cell interface through hemi-channel) are more important to support spermatogenesis instead of functional Cx43-based GJ channels between germ cells. Besides this genetic model, we also noted that adult rats treated with a single acute dose of adjudin, 1-(2,4-dichlorobenzyl)-1H-indazole-3-carbohydrazide, at 250 mg/kg b.w. via oral gavage, was found to induce irreversible male infertility due to permanent BTB disruption [120]. Interestingly, the testes in these rats also displayed a phenotype similar to Sertoli cell-specific Cx43 KO mice except that Sertoli cells in these rats were not mitotically active and a considerable down-regulation and disruptive localization of Cx43 were found [121]. While the population of spermatogonia was unaffected in these rats, spermatogonia failed to restart spermatogenesis due to meiotic arrest since spermatogonia could not differentiate into preleptotene spermatocytes [120,121]. Furthermore, F-actin organization in the seminiferous epithelium of these rats was grossly disrupted [121]. However, overexpression of Cx43 in the testis of adjudin treated rats at acute dose was able to reboot spermatogenesis since meiosis was detected in many of the affected tubules, but spermatids failed to undergo complete spermiogenesis [121]. Nonetheless, in tubules that had signs of spermatogenesis with meiosis, F-actin organization in the epithelium was re-established, making them in-distinguishable from control/normal tubules [121]. These findings are important since they illustrate the crucial role of Cx43-based GJ to support normal F-actin organization. Based on the study that a knockdown of Rictor function would impede GJ-channel communication [70], it is tempting to speculate that the homeostasis of F-actin organization in the seminiferous epithelium during the epithelial cycle is supported by chemical signals mediated by intercellular GJ-based channels with the intimate involvement of mTORC2. In fact, earlier studies have also demonstrated the role of GJs in organizing other cell junctions such as desmosome, TJ and basal ES at the immunological barrier to maintain BTB dynamics (i.e., assembly, disassembly and stabilization) [93,122].

Additionally, a recent study using a conditional knockout approach to delete mTOR in Sertoli cells using *mTOR*$^{flox/flox}$;*Amhr2-Cre* mice has shown that these mice are infertile, associated with considerable disorganization of seminiferous epithelium including a loss of Sertoli cell polarity, an increase in germ cell apoptosis, defects in germ cell adhesion that lead to specific loss of pachytene spermatocytes and spermatids [123]. In short, spermatocytes fail to advance beyond pachytene spermatocytes to enter meiosis I/II. However, specific KO of mTOR in Sertoli cells in *mTOR*$^{flox/flox}$;*Amhr2-Cre* mice did not appear to affect the basal ES/BTB function [123] since no apical ES could be found in these adult mice due to the absence of elongating/elongated spermatids. However, due the lack of data based on electron microscopy and the use of a functional BTB integrity assay in this report [123], it remains to be determined if the BTB integrity of these mice were in fact compromised. Interestingly, Sertoli cell-specific deletion of mTOR also leads to a considerable increase in p-rpS6 and mis-localization of Cx43 so that Cx43 no longer localizes at the base of the epithelium near the BTB but diffusively distributed across the entire epithelium [123]. Findings from this genetic model thus support the notion that mTOR is involved in GJ dynamics such as in the Rictor knockdown model by perturbing mTORC2 function which in turn perturbs GJ communication function [70]. In this context, it is of interest to note that since studies in other mammalian cells and/or epithelia have demonstrated the transport of miRNAs across GJs to modulate (or coordinate) cellular function [124–129], and germ cells are known to express a large number of miRNAs [130,131], it is thus likely that GJ in the testis including Cx43-based hemichannels may be used to modulate Sertoli cell function by germ cells through the transport of miRNAs under the influence of mTORC2. In short, mTORC2 may work with other signaling molecules and/or pathways downstream such as Akt1/2 to modulate GJ communication function, which in turn determines the transport of miRNAs into the Sertoli cell to regulate cytoskeletal function as schematically shown in Fig. 2. This possibility must be carefully evaluated in future studies.

4. FAK: p-FAK-Tyr407 *versus* p-FAK-Tyr397 on BTB dynamics

4.1. Introduction

FAK (focal adhesion kinase, also known as PTK2, protein tyrosine kinase 2, a 125 kDa polypeptide), a substrate of Src, is an extensively studied signal transducer downstream of integrin receptors that transmit signals at the cell-ECM (extracellular matrix) interface called focal adhesion complex (FAC or focal contact) to modulate epithelial and/or endothelial cell function [67,132–135]. In short, FAK is an important signaling

molecule at the FAC, an actin-based anchoring junction at the cell-matrix interface. However, there are no actin-based focal adhesion ultrastructures in the testis except the intermediate filament-based cell-matrix anchoring junction called hemidesmosome [51]. There are six putative phosphorylation sites along the FAK polypeptide: at Tyr-397, -407, -576, -577, -861 and -925 [67,136]. Tyr-397 is the auto-phosphorylation site of FAK, involved in its initial activation, and when it is phosphorylated (i.e., activated), this promotes Src binding, leading to conformational activation of Src and results in a dual-activated FAK-Src signaling complex [137]. On the other hand, FAK-Tyr407 phosphorylation suppresses Tyr397 phosphorylation to negatively regulate the overall function of FAK regarding its intrinsic kinase and biological activities, such as contact inhibition and cell cycle arrest [138], illustrating the antagonistic effects of p-FAK-Tyr397 *vs.* p-FAK-Tyr407. Interestingly, FAK was first detected in 2001 in adult rat testes at the basal ES/BTB site (in stage I–XIV tubules) but apparently limited to the cytoplasm of early spermatogenic cells, and also at the apical ES (mostly in stage IV–VIII tubules) using an anti-FAK monoclonal antibody by immunofluorescence microcopy [84]. Subsequent studies by immunofluorescence microcopy and/or immunohistochemistry, and co-immunoprecipitation using multiple antibodies from different sources have shown that FAK is an integrated component of the ES in 2003 which is a testis-specific actin-based cell-cell atypical adherens junction [139]. For instance, FAK is mostly expressed by the basal ES/BTB, but p-FAK-Tyr397 was predominantly localized at the apical ES and virtually undetectable at the basal ES/BTB [139,140]. In fact, the expression of p-FAK-Tyr397 persisted at the apical ES at the Sertoli-step 19 spermatid interface until the time sperm are released into the tubule lumen at spermiation [68,140], thus p-FAK-Tyr397 has been implicated to be involved in spermiation [141]. It is now known that p-FAK-Tyr397 forms a multi-protein signaling complex with β1-integrin, p130Cas, DOCK180, RhoA, vinculin, PI 3-kinase, and PKB, possibly creating a signaling platform at the apical ES to regulate spermiation [141–143]. Interestingly, FAK nor any of its activated forms is detected at the focal adhesion complex (or focal contacts, a cell-ECM (extracellular matrix) anchoring junction type) at the Sertoli cell-basement membrane interface (note: basement membrane is a modified form of ECM in the testis [86,144]). Subsequent studies have shown that, unlike p-FAK-Tyr397, p-FAK-Tyr407 is localized at the apical ES *and* also basal ES/BTB, but not at the basement membrane [68]. These observations in the testis are important because they illustrate the significance of FAK, an FAC protein found in virtually all epithelia, in adherens junctions since virtually all the earlier studies focus on the role of FAK as a cell-ECM regulator. In fact, it is now known that FAK, in particular p-FAK-Tyr397 is an important regulator of spermiation [141]. Studies using different constitutively active phosphomimetic mutants of FAK, such as p-FAK-Y397E and p-FAK-Y407E *vs.* constitutively inactive (i.e., non-phosphorylatable) phosphomimetic mutants of FAK, such as p-FAK-Y397F and p-FAK-Y407F to overexpress either in Sertoli cells cultured in vitro that mimic the Sertoli cell BTB or in the testis *in vivo* have shown that p-FAK-Tyr397 and p-FAK-Tyr407 have antagonistic effects on the Sertoli cell barrier in which p-FAK-Tyr397 promotes BTB remodeling by making its "leaky" whereas p-FAK-Tyr407 promotes BTB integrity by making it "tighter". These findings also suggest that, similar to mTORC1 and mTORC2, p-FAK-Tyr397 and p-FAK-Tyr407 serve as molecular "switches" to induce BTB disassembly or reassembly (Fig. 3).

4.2. p-FAK-Tyr397 promotes BTB remodeling vs. p-FAK-Tyr407 which promotes BTB integrity

Studies using various p-FAK mutants have shown that p-FAK-Tyr397 promotes BTB remodeling by making the Sertoli cell TJ-barrier "leaky". For instance, overexpression of p-FAK-Y397F (a non-phosphorylatable/constitutively inactive p-FAK-Tyr397 mutant) or p-FAK-Y407E (a constitutively active p-FAK-Tyr407 mutant) in Sertoli cell epithelium both promotes the integrity of Sertoli cell TJ-barrier, making it "tighter" [68]. However, overexpression of p-FAK-Y407F (a non-phosphorylatable/constitutively inactive p-FAK-Tyr407 mutant) or p-FAK-Y397E (a constitutively active p-FAK-Tyr397 mutant) promotes the Sertoli cell TJ-barrier remodeling, making it "leaky" [68]. Taken collectively, these findings are important since they illustrate that changes in the spatiotemporal expression of these two p-FAK proteins at the BTB microenvironment can possibly induce the immunological barrier to become either "leaky" or "tighter" at specific domains, such as at the site above or below the preleptotene spermatocytes connected in clones being transported across the BTB. In short, by increasing the level of p-FAK-Tyr407 and/or reducing the level of p-FAK-Tyr397 at the BTB microenvironment, this promotes BTB integrity by tightening the immunological barrier in non-stage VIII tubules (Fig. 3A). These changes also facilitate the establishment of a "new" BTB, such as at the rear end of the preleptotene spermatocytes during the transport of these germ cells across the immunological barrier (Fig. 3). On the other hand, by reducing the level of p-FAK-Tyr407 and/or increasing the level of p-FAK-Tyr397 at the BTB microenvironment promotes BTB remodeling by making the barrier "leaky" in stage VIII tubules (Fig. 3B). Studies have also shown that p-FAK-Tyr407, which is crucial to maintain BTB integrity, is likely working in concert with other regulatory proteins to modulate BTB dynamics (Fig. 3C). This notion is supported by use of a different animal study model, in which a biologically active peptide called F5-peptide was administered to the testis *in vivo,* and was shown to induce BTB remodeling, concomitant with a considerable down-regulation of the expression of p-FAK-Tyr407 in the testis [145]. Studies have shown that F5-peptide produced in the testis at the apical ES during spermiation is through activation of MMP-2 that cleaves laminin-γ3 chains [87,145–147]. The laminin-γ3 chain breakdown is essential for the release of sperm at spermiation since laminin-γ3 is also an integrated component of the apical ES [85, 87,88]. For instance, laminin-γ3 forms a functional ligand with laminin-α3 and laminin-β3 chains and this trimeric laminin ligand [88], also an elongated spermatid-based apical ES protein, in turn interacts with the Sertoli cell-specific α6β1-integrin receptor to create a bona fide adhesion complex at the apical ES [87,88]. A recent report has shown that the release of biologically active F5-peptide from the laminin-γ3 chain at the apical ES promotes further ES breakdown to facilitate the release of sperm at spermiation [147]. However, F5-peptide also considerably perturbs the spatial expression of p-FAK-Tyr407 since its expression at the basal ES/BTB during F5-peptide induced BTB disruption was found to be considerably down-regulated [145]. For instance, p-FAK-Tyr407 no longer prominently localized at the BTB near the basement membrane in the seminiferous epithelium when the BTB was found to be "leaky" based on an *in vivo* integrity assay [145]. These findings are important, since it demonstrates that p-FAK-Tyr407 is being used by the testis to promote F5-peptide-induced BTB restructuring, illustrating the physiological relevance of the earlier study using phosphomimetic mutants of p-FAKs [68]. Based on these findings, we now propose a model shown in Fig. 3C, which is similar to the model depicted in Fig. 2, illustrating that the mTORC1 and p-FAK-Tyr397 may be working in concert to promote BTB remodeling, by making the immunological barrier "leaky", such as at stage VIII of the epithelial cycle to support the transport of preleptotene spermatocytes across the immunological barrier. On the other hand, mTORC2 and p-FAK-Tyr407 may also be working in concert to promote BTB integrity, making the immunological barrier "tighter", such as in non-stage VIII tubules (Figs. 2 and 3). In this context, it is of interest to note that while the events of apical ES degeneration to support the release of sperm at spermiation *vs.* remodeling of the BTB to support preleptotene spermatocyte transport take place at the opposite ends of the seminiferous epithelium – at the apical (adluminal) near the tubule lumen *vs.* the site near the basement membrane – these two events are still functionally connected within a single Sertoli cell (or a single layer of Sertoli cells in the seminiferous epithelium) through integrin-based signaling function. At present, the signaling molecule(s) that elicit

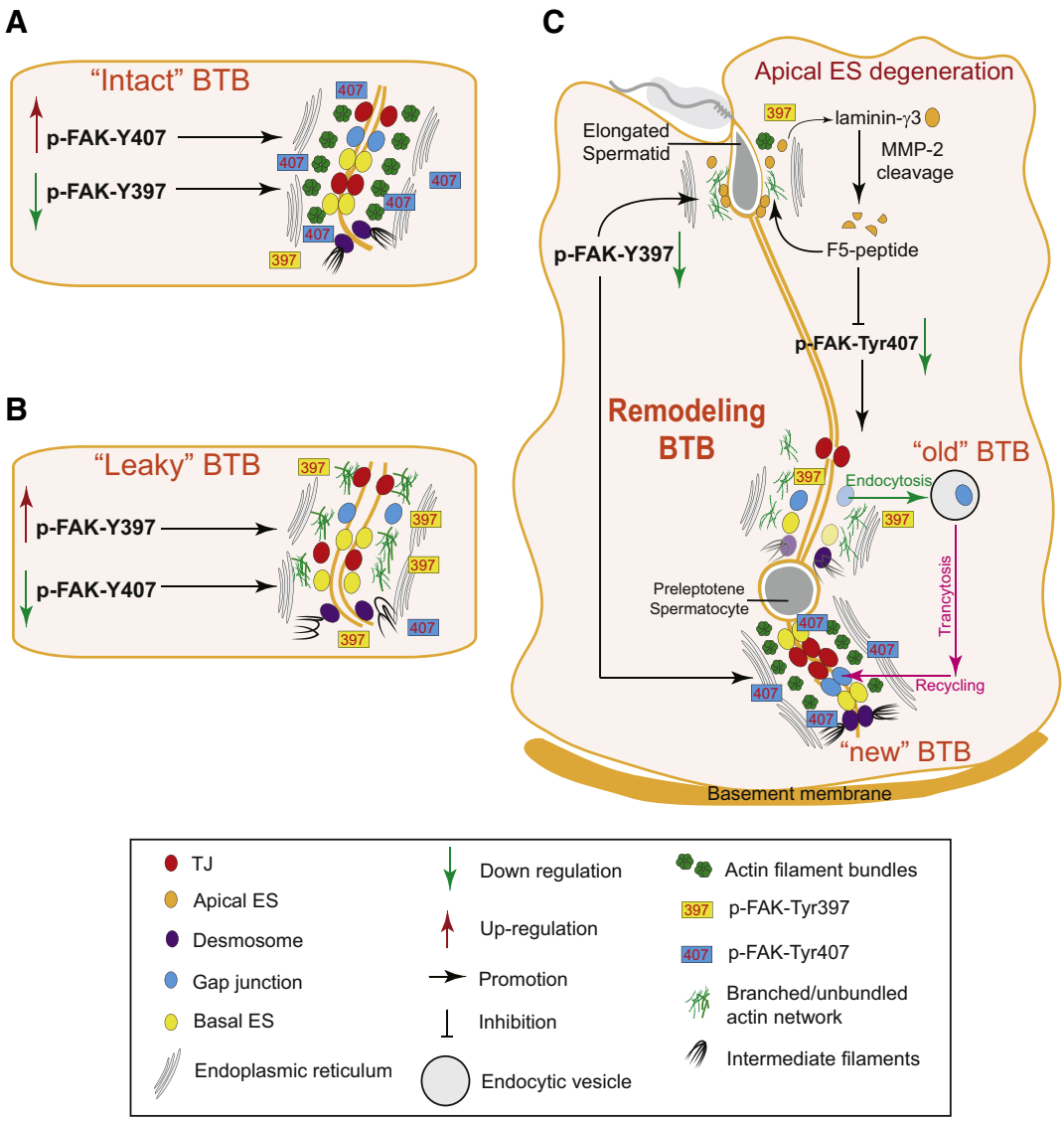


Fig. 3. A schematic diagram illustrating the involvement of p-FAK-Tyr397 and p-FAK-Tyr407 in BTB dynamics. p-FAK-Tyr397 and p-FAK-Tyr407 are the phosphorylated/activated form of FAK known to (A) promote BTB remodeling and (B) support BTB integrity, respectively. (C) These two signaling molecules work as molecular switches that turn "on" and "off" the immunological barrier by making it either "leaky" or "tighter" across the microdomains of the BTB to support the transport of preleptotene spermatocytes across the immunological barrier. Studies have shown that p-FAK-Tyr407 is also working in concert with F5-peptide generated at the apical ES just shortly before spermiation takes place to modulate the disassembly of the "old" BTB and the assembly of the "new" BTB above and below the preleptotene spermatocytes being transported across the immunological barrier. See text for further details.

these events remain unclear, but studies in other epithelia have shown that this likely involves integrin-based signaling molecules [148–150]. Studies using the F5-peptide model has also shown that F5-peptide likely exerts its effects, possibly mediated by a down-regulation on the expression of p-FAK-Tyr407, through inside-out and outside-in signaling in the testis [145], similar to other epithelia [151–153].

5. Concluding remarks and future perspectives

As briefly summarized above, we have critically evaluated findings based on studies at the BTB in rodents, illustrating that mTORC1 and p-FAK-Tyr397 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". In short, 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 as molecular switches to turn "off" or "on" the immunological barrier, thereby supporting the transport of preleptotene spermatocytes across the barrier during spermatogenesis. However, it is not known at present the molecules and or signaling pathways that connect mTORC1 and mTORC2 with p-FAK-Tyr397 and p-FAK-Tyr407 to work in concert to modulate BTB dynamics. 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 [154–156] 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 [157] 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 non-targeting shRNA was shown to perturb the Sertoli cell TJ-barrier function via its disruptive effects on F-actin organization in Sertoli cells [157,158]. More important, the laminin α2 knockdown was associated with an up-regulation of p-rpS6 expression [158], 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 [103], was found to block the laminin α2 shRNA-mediated disruptive effects on F-actin organization in Sertoli cells [158]. 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 [146] may be utilizing FAK to regulate BTB function. It has been shown that F5-peptide, a biologically active 50-amino acid residue 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 [145]. 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 [145,147]. 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 [145]. 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 spermatocytes 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 [157,158] or F5-peptide from the laminin-γ3 chain at the apical ES [145–147] as briefly discussed above. In fact, this possibility is also supported in a recent report based on a study *in vitro* wherein germ cells cocultured with Sertoli cells are found to modulate Sertoli TJ-permeability barrier function [159]. On the other hand, proteins that are expressed at the apical ES and TJ as well as small GTPases can also modulate the barrier function as shown in other epithelia and/or endothelia as recently reviewed [7,160,161]. Emerging evidence has also supported the involvement of Frizzled receptors in BBB formation and maintenance [162]. Collectively, the findings reviewed herein have supported our beliefs that the hypothetical model depicted in Figs. 2 and 3 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 barriers.

Declaration of conflicts of interest

Nothing to declare.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

This work was supported by grants from the National Institutes of Health (R01 HD056034 to C.Y.C. and U54 HD029990 Project 5 to C.Y.C.); National Natural Science Foundation of China (NSFC) (31371176 to X.X.), Qianjiang Talents Program (QJD1502029 to X.X.), Zhejiang Province Funding (Science Technology Department, 2016F10010 to X.X.); Hong Kong Research Grants Council (RGC) General Research Fund (GRF) (771513 to W.M.L.), NSFC/RGC Joint Research Scheme (N_HKU 717/12 to W.M.L.), and University of Hong Kong CRCG Seed Funding (to W.M.L.); and Hong Kong Baptist University Strategy Development Fund (SDF11-1215-P07 to C.K.C.W.).

References

[1] A.S. Easton, Regulation of permeability across the blood–brain barrier, Adv. Exp. Med. Biol. 763 (2012) 1–19.
[2] S.C. Nalle, J.R. Turner, Endothelial and epithelial barriers in graft-verus-host disease, Adv. Exp. Med. Biol. 763 (2012) 105–131.
[3] C.Y. Cheng, D.D. Mruk, The blood–testis barrier and its implication in male contraception, Pharmacol. Rev. 64 (2012) 16–64.
[4] P.G. Stanton, Regulation of the blood–testis barrier, Semin. Cell Dev. Biol. 59 (2016) 166–173.
[5] T. Ashraf, O. Kis, N. Banerjee, R. Bendayan, Drug transporters at brain barriers: expression and regulation by neurological disorders, Adv. Exp. Med. Biol. 763 (2012) 20–69.
[6] D.D. Mruk, C.Y. Cheng, The mammalian blood–testis barrier: its biology and regulation, Endocr. Rev. 36 (2015) 564–591.
[7] Y.A. Komarova, K. Kruse, D. Mehta, A.B. Malik, Protein interactions at endothelial juunctions and signaling mechanisms regulating endothelial permeability, Circ. Res. 120 (2017) 179–206.
[8] A. Fasano, Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer, Physiol. Rev. 91 (2011) 151–175.
[9] M.A. Odenwald, J.R. Turner, The intestinal epithelial barrier: a therapeutic target? Nat. Rev. Gastroenterol. Hepatol. (2016) (advance online publication).
[10] M.P. Greenbaum, T. Iwamori, G.M. Buchold, M.M. Matzuk, Germ cell intercellular bridges, Cold Spring Harb. Perspect. Biol. 3 (2011) a005850.
[11] L. Hermo, R.M. Pelletier, D.G. Cyr, C.E. Smith, Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells. Part 4: intercellular bridges, mitochondria, nuclear envelope, apoptosis, ubiquitination, membrane/voltage-gated channels, methylation/acetylation, and transcription factors, Microsc. Res. Tech. 73 (2010) 364–408.
[12] X. Xiao, D.D. Mruk, C.K.C. Wong, C.Y. Cheng, Germ cell transport across the seminiferous epithelium during spermatogenesis, Physiology 29 (2014) 286–298.
[13] E.I. Tang, D.D. Mruk, C.Y. Cheng, Regulation of microtubule (MT)-based cytoskeleton in the seminiferous epithelium during spermatogenesis, Semin. Cell Dev. Biol. 59 (2016) 35–45.
[14] R.A. Hess, L.R. de Franca, Spermatogenesis and cycle of the seminiferous epithelium, Adv. Exp. Med. Biol. 636 (2008) 1–15.
[15] L. Hermo, R.M. Pelletier, D.G. Cyr, C.E. Smith, Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells. Part 1: background to spermatogenesis, spermatogonia, and spermatocytes, Microsc. Res. Tech. 73 (2010) 241–278.
[16] N. Li, E.I. Tang, C.Y. Cheng, Regulation of blood–testis barrier by actin binding proteins and protein kinases, Reproduction 151 (2016) R29–R41.
[17] O. Kis, K. Robillard, G.N. Chan, R. Bendayan, The complexities of antiretroviral drug–drug interactions: role of ABC and SLC transporters, Trends Pharmacol. Sci. 31 (2010) 22–35.
[18] K.R. Robillard, M.T. Hoque, R. Bendayan, Expression of ATP-binding cassette membrane transporters in rodent and human Sertoli cells: relevance to the permeability of antiretroviral therapy at the blood–testis barrier, J. Pharmacol. Exp. Ther. 340 (2012) 96–108.
[19] J. Konig, F. Muller, M.F. Fromm, Transporters and drug–drug interactions: important determinants of drug disposition and effects, Pharmacol. Rev. 65 (2013) 944–966.
[20] D.D. Mruk, L. Su, C.Y. Cheng, Emerging role for drug transporters at the blood–testis barrier, Trends Pharmacol. Sci. 32 (2011) 99–106.
[21] A. Le Tortorec, N. Dejucq-Rainsford, The male genital tract: a host for HIV, Gynecol. Obstet. Fertil. 35 (2007) 1245–1250.
[22] L. Houzet, G. Matusali, N. Dejucq-Rainsford, Origins of HIV-infected leukocytes and virions in semen, J. Infect. Dis. 210 (Suppl. 3) (2014) S622–S630.
[23] J. Govero, P. Esakky, S.M. Scheaffer, E. Fernandez, A. Drury, D.J. Platt, M.J. Gorman, J.M. Richner, E.A. Caine, V. Salazar, K.H. Moley, M.S. Diamond, Zika virus infection damages the testes in mice, Nature 540 (2016) 438–442.
[24] M.A. Jenabian, C.T. Costiniuk, V. Mehraj, F.M. Ghazawi, R. Fromentin, J. Brousseau, P. Brassard, M. Belanger, P. Ancuta, R. Bendayan, N. Chomont, J.P. Routy, g. Orchid study, Immune tolerance properties of the testicular tissue as a viral sanctuary site in ART-treated HIV-infected adults, AIDS 30 (2016) 2777–2786.
[25] M.M. Al-Obaidi, A. Bahadoran, L.S. Har, W.S. Mui, J. Rajarajeswaran, K. Zandi, R. Manikam, S.D. Sekaran, Japanese encephalitis virus disrupts blood–brain barrier and modulates apoptosis proteins in THBMEC cells, Virus Res. 233 (2017) 17–28.
[26] R. Shi, M.H. Soomro, R. She, Y. Yang, T. Wang, Q. Wu, H. Li, W. Hao, Evidence of Hepatitis E virus breaking through the blood–brain barrier and replciating in the central nervous system, J. Viral Hepat. 23 (2016) 930–939.
[27] M. McRae, HIV and viral protein effects on the blood–brain barrier, Tissue Barriers 4 (2016) e1143543.

[28] E. Clark, B. Nava, M. Caputi, Tat is a multifunctional viral protein that modulates cellular gene expression and functions, Oncotarget (2017) (in press) 10.18632/oncotarget.15174 PMID 28187438.
[29] K.R. Spindler, T.H. Hsu, Viral disruption of the blood–brain barrier, Trends Microbiol. 20 (2012) 282–290.
[30] J.P. Louboutin, D.S. Strayer, Blood–brain barrier abnormalities caused by HIV-1 gp120: mechanistic and therapeutic implications, TheScientificWorldJOURNAL 2012 (2012) 482575.
[31] D.D. Mruk, C.Y. Cheng, Drug transporters in spermatogenesis: a re-evaluation of recent data on P-glycoprotein, Spermatogenesis 2 (2012) 70–72.
[32] L. Su, D.D. Mruk, W.Y. Lui, W.M. Lee, C.Y. Cheng, P-glycoprotein regulates blood–testis barrier dynamics via its effects on the occludin/zonula occludens 1 (ZO-1) protein complex mediated by focal adhesion kinase (FAK), Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 19623–19628.
[33] K.R. Robillard, G.N. Chan, G. Zhang, C.C. la Porte, W.R. Bendayan, Role of P-glycoprotein in the distribution of the HIV protease inhibitor atazanavir in the brain and male genital tract, Antimicrob. Agents Chemother. 58 (2014) (1713-1722).
[34] G. Darcis, R.W. Coombs, C. Van Lint, Exploring the anatomical HIV reservoirs: role of the testicular tissue, AIDS 30 (2016) 2891–2893.
[35] R.W. Coombs, D. Lockhart, S.O. Ross, L. Deutsch, J. Dragavon, K. Diem, T.M. Hooton, A.C. Collier, L. Corey, J.N. Krieger, Lower genitourinary tract sources of seminal HIV, J. Acquir. Immune Defic. Syndr. 41 (2006) 430–438.
[36] A.F. Fois, B.J. Brew, The potential of the CNS as a reservoir for HIV-1 infection: implicaitons for HIV eradication, Curr. HIV/AIDS Rep. 12 (2015) 299–303.
[37] G. Kaur, L.A. Thompson, J.M. Dufour, Sertoli cells — immunological sentinels of spermatogenesis, Semin. Cell Dev. Biol. 30 (2014) 36–44.
[38] G. Kaur, C.R. Long, J.M. Dufour, Genetically engineered immune privileged Sertoli cells — a new road to cell based gene therapy, Spermatogenesis 2 (2012) 23–31.
[39] L.R. Franca, R.A. Hess, J.M. Dufour, M.C. Hofmann, M.D. Griswold, The Sertoli cell: one hundred fifty years of beauty and plasticity, Andrology 4 (2016) 189–212.
[40] K. Wright, R. Dziuk, P. Mital, G. Kaur, J.M. Dufour, Xenotransplanted pig Sertoli cells inhibit both the alternative and classical pathways of complement mediated cell lysis while pig islets are killed, Cell Transplant. (2016).
[41] H.P. Selawry, D.F. Cameron, Sertoli cell-enriched fractions in successful islet cell transplantation, Cell Transplant. 2 (1993) 123–129.
[42] H.P. Selawry, M. Kotb, H.G. Herrod, Z.N. Lu, Production of a factor, or factors, suppressing IL-2 production and T cell proliferation by Sertoli cell-enriched preparations. A potential role for islet transplantation in an immunologically privileged site, Transplantation 52 (1991) 846–850.
[43] Q. Chen, T. Deng, D. Han, Testicular immunoregulation and spermatogenesis, Semin. Cell Dev. Biol. 59 (2016) 157–165.
[44] Y. Huang, M.T. Hoque, M.A. Jenabian, K. Vyboh, S.K. Whyte, N.L. Sheehan, P. Brassard, M. Belanger, N. Chomont, C.V. Fletcher, J.P. Routy, R. Bendayan, Antiretroviral drug transporters and metabolic enzymes in human testicular tissue: potential contribution to HIV-1 sanctuary site, J. Antimicrob. Chemother. 71 (2016) 1954–1965.
[45] X. Qian, Y.H. Cheng, D.D. Mruk, C.Y. Cheng, Breast cancer resistance protein (Bcrp) and the testis — an unexpected turn of events, Asian J. Androl. 15 (2013) 455–460.
[46] E. Sykova, C. Nicholson, Diffusion in brain extracellular space, Physiol. Rev. 88 (2008) 1277–1340.
[47] R. Daneman, L. Zhou, A.A. Kebede, B.A. Barres, Pericytes are required for blood–brain barrier integrity during embryogenesis, Nature 468 (2010) 562–566.
[48] A. Armulik, G. Genove, M. Mae, M.H. Nisancioglu, E. Wallgard, C. Niaudet, L. He, J. Norlin, P. Lindblom, K. Strittmatter, B.R. Johansson, C. Betsholtz, Pericytes regulate the blood–brain barrier, Nature 468 (2010) 557–561.
[49] A.W. Vogl, K.S. Vaid, J.A. Guttman, The Sertoli cell cytoskeleton, Adv. Exp. Med. Biol. 636 (2008) 186–211.
[50] B.P. Setchell, Blood–testis barrier, functional and transport proteins and spermatogenesis, Adv. Exp. Med. Biol. 636 (2008) 212–233.
[51] C.Y. Cheng, D.D. Mruk, Cell junction dynamics in the testis: Sertoli-germ cell interactions and male contraceptive development, Physiol. Rev. 82 (2002) 825–874.
[52] R.M. Pelletier, The blood–testis barrier: the junctional permeability, the proteins and the lipids, Prog. Histochem. Cytochem. 46 (2011) 49–127.
[53] R. Pelletier, S. Byers, The blood–testis barrier and Sertoli cell junctions: structural considerations, Microsc. Res. Tech. 20 (1992) 3–33.
[54] M. Dym, D.W. Fawcett, The blood–testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium, Biol. Reprod. 3 (1970) 308–326.
[55] M. Gregory, D.G. Cyr, The blood–epididymis barrier and inflammation, Spermatogenesis 4 (2014) e979619.
[56] J.M. Wells, R.J. Brummer, M. Derrien, T.T. MacDonald, F. Troost, P.D. Cani, V. Theodorou, J. Dekker, A. Meheust, W.M. de Vos, A. Mercenier, A. Nauta, C.L. Garcia-Rodenas, Homeostasis of the gut barrier and potential biomarkers, Am. J. Physiol. Gastrointest. Liver Physiol. 312 (2017) G171–G193.
[57] L.D. Russell, Observations on the inter-relationships of Sertoli cells at the level of the blood–testis barrier: evidence for formation and resorption of Sertoli-Sertoli tubulobulbar complexes during the spermatogenic cycle of the rat, Am. J. Anat. 155 (1979) 259–279.
[58] L.D. Russell, The blood–testis barrier and its formation relative to spermatocyte maturation in the adult rat: a lanthanum tracer study, Anat. Rec. 190 (1978) 99–112.
[59] L.D. Russell, R.N. Peterson, Sertoli cell junctions: morphological and functional correlates, Int. Rev. Cytol. 94 (1985) 177–211.
[60] K.I. Hosoya, M. Tachikawa, The inner blood–retinal barrier: molecular structure and transport biology, Adv. Exp. Med. Biol. 763 (2012) 85–104.
[61] L.R. Franca, S.A. Auharek, R.A. Hess, J.M. Dufour, B.T. Hinton, Blood–tissue barriers: morphofunctional and immunological aspects of the blood–testis and blood–epididymal barriers, Adv. Exp. Med. Biol. 763 (2012) 237–259.
[62] C.Y. Cheng, D.D. Mruk, A local autocrine axis in the testes that regulates spermatogenesis, Nat. Rev. Endocrinol. 6 (2010) 380–395.
[63] D.D. Mruk, C.Y. Cheng, Cell–cell interactions at the ectoplasmic specialization in the testis, Trends Endocrinol. Metab. 15 (2004) 439–447.
[64] D.D. Mruk, C.Y. Cheng, Sertoli–Sertoli and Sertoli–germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis, Endocr. Rev. 25 (2004) 747–806.
[65] N. Li, C.Y. Cheng, Mammalian target of rapamycin complex (mTOR) pathway modulates blood–testis barrier (BTB) function through F-actin organization and gap junction, Histol. Histopathol. 11753 (2016).
[66] K.W. Mok, D.D. Mruk, C.Y. Cheng, Regulation of blood–testis barrier (BTB) dynamics during spermatogenesis via the "Yin" and "Yang" effects of mammalian target of rapamycin complex 1 (mTORC1) and mTORC2, Int. Rev. Cell Mol. Biol. 301 (2013) 291–358.
[67] C.Y. Cheng, D.D. Mruk, Regulation of blood–testis barrier dynamics by focal adhesion kinase (FAK). An unexpected turn of events, Cell Cycle 8 (2009) 3493–3499.
[68] P.P.Y. Lie, D.D. Mruk, K.W. Mok, L. Su, W.M. Lee, C.Y. Cheng, Focal adhesion kinase-Tyr^{407} and -Tyr^{397} exhibit antagonistic effects on blood–testis barrier dynamics in the rat, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 12562–12567.
[69] K.W. Mok, D.D. Mruk, B. Silvestrini, C.Y. Cheng, rpS6 regulates blood–testis barrier dynamics by affecting F-actin organization and protein recruitment, Endocrinology 153 (2012) 5036–5048.
[70] K.W. Mok, D.D. Mruk, W.M. Lee, C.Y. Cheng, Rictor/mTORC2 regulates blood–testis barrier dynamics via its effects on gap junction communications and actin filament network, FASEB J. 27 (2013) 1137–1152.
[71] K.W. Mok, D.D. Mruk, C.Y. Cheng, rpS6 regulates blood–testis barrier dynamics through Akt-mediated effects on MMP-9, J. Cell Sci. 127 (2014) 4870–4882.
[72] K.W. Mok, H. Chen, W.M. Lee, C.Y. Cheng, rpS6 regulates blood–testis barrier dynamics through Arp3-mediated actin microfilament organization in rat Sertoli cells. An in vitro study, Endocrinology 156 (2015) 1900–1913.
[73] D.D. Sarbassov, S.M. Ali, D.H. Kim, D.A. Guertin, R.R. Latek, H. Erdjument-Bromage, P. Tempst, D.M. Sabatini, Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton, Curr. Biol. 14 (2004) 1296–1302.
[74] R.P. Amann, The cycle of the seminiferous epithelium in humans: a need to revisit? J. Androl. 29 (2008) 469–487.
[75] M. Parvinen, Regulation of the seminiferous epithelium, Endocr. Rev. 3 (1982) 404–417.
[76] D.G. de Rooij, The spermatogonial stem cell niche, Microsc. Res. Tech. 72 (2009) 580–585.
[77] L.D. Russell, Observations on rat Sertoli ectoplasmic ('junctional') specializations in their association with germ cells of the rat testis, Tissue Cell 9 (1977) 475–498.
[78] C.Y. Cheng, D.D. Mruk, Biochemistry of Sertoli cell/germ cell junctions, germ cell transport, and spermiation in the seminiferous epithelium, in: M.D. Griswold (Ed.), Sertoli Cell Biology, 2nd edition.Elsevier, Amsterdam 2015, pp. 333–383, http://dx.doi.org/10.1016/B978-0-12-417047-6.00012.0.
[79] K. Ozaki-Kuroda, H. Nakanishi, H. Ohta, H. Tanaka, H. Kurihara, S. Mueller, K. Irie, W. Ikeda, T. Sakai, E. Wimmer, Y. Nishimune, Y. Takai, Nectin couples cell-cell adhesion and the actin scaffold at heterotypic testicular junctions, Curr. Biol. 12 (2002) 1145–1150.
[80] G. Gliki, K. Ebnet, M. Aurrand-Lions, B.A. Imhof, R.H. Adams, Spermatid differentiation requires the assembly of a cell polarity complex downstream of junctional adhesion molecule-C, Nature 431 (2004) 320–324.
[81] M. Salanova, M. Stefanini, I. De Curtis, F. Palombi, Integrin receptor α6β1 is localized at specific sites of cell-to-cell contact in rat seminiferous epithelium, Biol. Reprod. 52 (1995) 79–87.
[82] M. Salanova, G. Ricci, C. Boitani, M. Stefanini, S. De Grossi, F. Palombi, Junctional contacts between Sertoli cells in normal and aspermatogenic rat seminiferous epithelium contain α6β1 integrins, and their formation is controlled by follicle-stimulating hormone, Biol. Reprod. 58 (1998) 371–378.
[83] F. Palombi, M. Salanova, G. Tarone, D. Farini, M. Stefanini, Distribution of β1 integrin subunit in rat seminiferous epithelium, Biol. Reprod. 47 (1992) 1173–1182.
[84] D.J. Mulholland, S. Dedhar, A.W. Vogl, Rat seminiferous epithelium contains a unique junction (ectoplasmic specialization) with signaling properties both of cell/cell and cell/matrix junctions, Biol. Reprod. 64 (2001) 396–407.
[85] M. Koch, P.F. Olson, A. Albus, W. Jin, D.D. Hunter, W.J. Brunken, R.E. Burgeson, M.F. Champliaud, Characterization and expression of the laminin γ3 chain: a novel, non-basement membrane-associated, laminin chain, J. Cell Biol. 145 (1999) 605–618.
[86] M.K.Y. Siu, C.Y. Cheng, Dynamic cross-talk between cells and the extracellular matrix in the testis, Bioessays 26 (2004) 978–992.
[87] M.K.Y. Siu, C.Y. Cheng, Interactions of proteases, protease inhibitors, and the β1 integrin/laminin γ3 protein complex in the regulation of ectoplasmic specialization dynamics in the rat testis, Biol. Reprod. 70 (2004) 945–964.
[88] H.H.N. Yan, C.Y. Cheng, Laminin α3 forms a complex with β3 and γ3 chains that serves as the ligand for α6β1-integrin at the apical ectoplasmic specialization in adult rat testes, J. Biol. Chem. 281 (2006) 17286–17303.
[89] G. Pointis, J. Gilleron, D. Carette, D. Segretain, Testicular connexin 43, a precocious molecular target for the effect of environmental toxicants on male fertility, Spermatogenesis 1 (2011) 303–317.
[90] G. Pointis, D. Segretain, Role of connexin-based gap junction channels in testis, Trends Endocrinol. Metab. 16 (2005) 300–306.

[91] M. Risley, I. Tan, C. Roy, J. Saez, Cell-, age-, and stage-dependent distribution of connexin 43 gap junctions in testes, J. Cell Sci. 103 (1992) 81–96.
[92] D. Carette, K. Weider, J. Gilleron, S. Giese, J. Dompierre, M. Bergmann, R. Brehm, J.P. Denizot, D. Segretain, G. Pointis, Major involvement of connexin 43 in seminiferous epithelial junction dynamics and male fertility, Dev. Biol. 346 (2010) 54–67.
[93] M.W.M. Li, D.D. Mruk, W.M. Lee, C.Y. Cheng, Connexin 43 and plakophilin-2 as a protein complex that regulates blood–testis barrier dynamics, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 10213–10218.
[94] J.S. Young, J.A. Guttman, K.S. Vaid, A.W. Vogl, Tubulobulbar complexes are intercellular podosome-like structures that internalize intact intercellular junctions during epithelial remodeling events in the rat testis, Biol. Reprod. 80 (2009) 162–174.
[95] L.M. Domke, S. Rickelt, Y. Dorflinger, C. Kuhn, S. Winter-Simanowski, R. Zimbelmann, R. Rosin-Arbesfeld, H. Heid, W.W. Franke, The cell–cell junctions of mammalian testes: I. The adhering junctions of the seminiferous epithelium represent special differentiation structures, Cell Tissue Res. 357 (2014) 645–665.
[96] N.P.Y. Lee, D.D. Mruk, W.M. Lee, C.Y. Cheng, Is the cadherin/catenin complex a functional unit of cell–cell-actin-based adherens junctions (AJ) in the rat testis? Biol. Reprod. 68 (2003) 489–508.
[97] K. Johnson, K. Boekelheide, Dynamic testicular adhesion junctions are immunologically unique. II. Localization of classic cadherins in rat testis, Biol. Reprod. 66 (2002) 992–1000.
[98] M. Laplante, D.M. Sabatini, mTOR signaling in growth control and disease, Cell 149 (2012) 274–293.
[99] J. Bockaert, P. Marin, mTOR in brain physiology and pathologies, Physiol. Rev. 95 (2015) 1157–1187.
[100] L. Kassem, O. Abdel-Rahman, Targeting mTOR pathway in gynecological malignancies: biological rationale and systematic review of published data, Crit. Rev. Oncol. Hematol. 108 (2016) 1–12.
[101] L.C. Kim, R.S. Cook, J. Chen, mTORC1 and mTORC2 in cancer and the tumor microenvironment. (DOI:10.1038/onc.2016.363), Oncogene (2016).
[102] K. Maiese, Novel nervous and multi-system regenerative therapeutic strategies for diabetes mellitus with mTOR, Neural Regen. Res. 11 (2016) 372–385.
[103] R.A. Saxton, D.M. Sabatini, mTOR signaling in growth, metabolism, and disease, Cell 168 (2017) 960–976.
[104] M. Shimobayashi, M.N. Hall, Making new contacts: the mTOR network in metabolism and signalling crosstalk, Nat. Rev. Mol. Cell Biol. 15 (2014) 155–162.
[105] O. Meyuhas, Ribosomal protein S6 phosphorylation: four decades of research, Int. Rev. Cell Mol. Biol. 320 (2015) 41–73.
[106] H. Xu, L. Shen, X. Chen, Y.H. Ding, J.Q. He, J. Zhu, Y. Wang, X. Liu, mTOR/P70S6K promotes spermatogonia proliferation and spermatogenesis in Sprague Dawley rats, Reprod. Biomed. Online 32 (2016) 207–217.
[107] C. Schell, O. Kretz, W. Liang, B. Kiefer, S. Schneider, D. Sellung, T. Bork, C. Leiber, M.A. Ruegg, C. Mallidis, S. Schlatt, A. Mayerhofer, T.B. Huber, F. Grahammer, The rapamycin-sensitive complex of mammalian target of rapamycin is essential to maintain male fertility, Am. J. Pathol. 186 (2016) 324–336.
[108] K. Huang, D.C. Fingar, Growing knowledge of the mTOR signaling network, Semin. Cell Dev. Biol. 36 (2014) 79–90.
[109] S. Rotkopf, Y. Hamberg, T. Aigaki, S.B. Snapper, B.Z. Shilo, E.D. Shejter, The WASp-based actin polymerization machinery is required in somatic support cells for spermatid maturation and release, Development 138 (2011) 2729–2739.
[110] X. Xiao, D.D. Mruk, E.I. Tang, R. Massarwa, K.W. Mok, N. Li, C.K. Wong, W.M. Lee, S.B. Snapper, B.Z. Shilo, E.D. Schejter, C.Y. Cheng, N-WASP is required for structural integrity of the blood–testis barrier, PLoS Genet. 10 (2014) e1004447.
[111] H. Dong, Z. Chen, C. Wang, Z. Xiong, W. Zhao, C. Jia, J. Lin, Y. Lin, W. Yuan, A.Z. Zhao, X. Bai, Rictor regulates spermatogenesis by controlling Sertoli cell cytoskeletal organization and cell polarity in the mouse testis, Endocrinology 156 (2015) 4244–4256.
[112] M. Risley, Connexin gene expression in seminiferous tubules of the Sprague–Dawley rat, Biol. Reprod. 62 (2000) 748–754.
[113] S.S.W. Chung, W.M. Lee, C.Y. Cheng, Study on the formation of specialized inter-Sertoli cell junctions in vitro, J. Cell. Physiol. 181 (1999) 258–272.
[114] G.M. Kidder, D.G. Cyr, Roles of connexins in testis development and spermatogenesis, Semin. Cell Dev. Biol. 50 (2016) 22–30.
[115] D. Chevallier, D. Carette, D. Segretain, J. Gilleron, G. Pointis, Connexin 43 a checkpoint component of cell proliferation implicated in a wide range of human testis disease, Cell. Mol. Life Sci. 70 (2013) 1207–1220.
[116] R. Brehm, M. Zeiler, C. Ruttinger, K. Herde, M. Kibschull, E. Winterhager, K. Willecke, F. Guillou, C. Lecureuil, K. Steger, L. Konrad, K. Biermann, K. Failing, M. Bergmann, A Sertoli cell-specific knockout of connexin43 prevents initiation of spermatogenesis, Am. J. Pathol. 171 (2007) 19–31.
[117] S. Giese, H. Hossain, M. Markmann, T. Chakraborty, S. Tchatalbachev, F. Guillou, M. Bergmann, K. Failing, K. Weider, R. Brehm, Sertoli-cell-specific knockout of connexin 43 leads to multiple alterations in testicular gene expression in prepubertal mice, Dis. Model. Mech. 5 (2012) 895–913.
[118] K. Weider, M. Bergmann, S. Giese, F. Guillou, K. Failing, R. Brehm, Altered differentiation and clustering of Sertoli cells in transgenic mice showing a Sertoli cell specific knockout of the connexin 43 gene, Differentiation 82 (2011) 38–49.
[119] S. Gunther, D. Fietz, K. Weider, M. Bergmann, R. Brehm, Effects of a murine germ cell-specific knockout of connexin 43 on connexin expression in testis and fertility, Transgenic Res. 22 (2013) 631–641.
[120] K.W. Mok, D.D. Mruk, W.M. Lee, C.Y. Cheng, Spermatogonial stem cells alone are not sufficient to re-initiate spermatogenesis in the rat testis following adjudin-induced infertility, Int. J. Androl. 35 (2012) 86–101.
[121] N. Li, D.D. Mruk, K.W. Mok, M.W. Li, C.K. Wong, W.M. Lee, D. Han, B. Silvestrini, C.Y. Cheng, Connexin 43 reboots meiosis and reseals blood–testis barrier following toxicant-mediated aspermatogenesis and barrier disruption, FASEB J. 30 (2016) 1436–1452.
[122] M.W.M. Li, D.D. Mruk, W.M. Lee, C.Y. Cheng, Connexin 43 is critical to maintain the homeostasis of blood–testis barrier via its effects on tight junction reassembly, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 17998–18003.
[123] A. Boyer, M. Girard, D.S. Thimmanahalli, A. Levasseur, C. Celeste, M. Paquet, R. Duggavathi, D. Boerboom, mTOR regulates gap junction alpha-1 protein trafficking in Sertoli cells and is required for the maintenance of spermatogenesis in mice, Biol. Reprod. 95 (2016) 13.
[124] G.S. Higa, E. de Sousa, L.T. Walter, E.R. Kinjo, R.R. Resende, A.H. Kihatra, MicroRNAs in neuronal communication, Mol. Neurobiol. 49 (2014) 1309–1326.
[125] H. Lemcke, G. Steinhoff, R. David, Gap junctional shuttling of miRNA — a novel pathway of intercellular gene regulation and its prospects in clinical application, Cell. Signal. 27 (2015) 2506–2514.
[126] L. Rimkute, V. Jotautis, A. Marandykina, R. Sveikatiene, I. Antanaviciute, V.A. Skeberdis, The role of neural connexins in HeLa cell mobility and intercellular communication through tunneling tubes, BMC Cell Biol. 17 (2016) 3.
[127] A.K. Gadok, D.J. Busch, S. Ferrati, B. Li, H.D. Smyth, J.C. Stachowiak, Connectosomes for direct molecular delivery to the cellular cytoplasm, J. Am. Chem. Soc. 138 (2016) 12833–12840.
[128] X. Hong, W.C. Sin, A.L. Harris, C.C. Naus, Gap junctions modualte glioma invasion by direct transfer of microRNA, Oncotarget 6 (2015) 15566–15577.
[129] V. Valiunas, H.Z. Wang, L. Li, C. Gordon, L. Valiuniene, I.S. Cohen, P.R. Brink, A comparison of two cellular delivery mechanisms for small interfering RNA, Physiol. Rep. 3 (2015).
[130] R. Song, G.W. Hennig, Q. Wu, C. Jose, H. Zheng, W. Yan, Male germ cells express abundant endogenous siRNAs, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 13159–131564.
[131] J. Wu, J. Bao, M. Kim, S.Y. Yuan, C. Tang, H. Zheng, G.S. Mastick, C. Xu, W. Yan, Two miRNA clusters, miR-34b/c and miR-449, are essential for normal brain development, motile ciliogenesis, and spermatogenesis, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) E2851–E2857.
[132] T. Boutros, E. Chevet, P. Metrakos, Mitogen-activated protein (MAP) kinase/MAP kinase phosphatase regulation: roles in cell growth, death, and cancer, Pharmacol. Rev. 60 (2008) 261–310.
[133] L. Pentassuglia, D.B. Sawyer, ErbB/integrin singaling interactions in regulation of myocardial cell–cell and cell–matrix interactions, Biochim. Biophys. Acta 1833 (2013) 909–916.
[134] V.P. Hytönen, B. Wehrle-Haller, Mechanosensing in cell–matrix adhesions — converting tension into chemical signals, Exp. Cell Res. 343 (2016) 35–41.
[135] E. Sackmann, How actin/myosin crosstalks guide the adhesion, locomotion and polarization of cells, Biochim. Biophys. Acta, Mol. Cell. Res. 1853 (2015) 3132–3142.
[136] S.K. Mitra, D.A. Hanson, D.D. Schlaepfer, Focal adhesion kinase: in command and control of cell motility, Nat. Rev. Mol. Cell Biol. 6 (2005) 56–68.
[137] D.D. Schlaepfer, S.K. Mitra, D. Ilic, Control of motile and invasive cell phenotypes by focal adhesion kinase, Biochim. Biophys. Acta 1692 (2004) 77–102.
[138] Y. Lim, H. Park, J. Jeon, I. Han, J. Kim, E.H. Jho, E.S. Oh, Focal adhesion kinase is negatively regulated by phosphorylation at tyrosine 407, J. Biol. Chem. 282 (2007) 10398–10404.
[139] M.K.Y. Siu, D.D. Mruk, W.M. Lee, C.Y. Cheng, Adhering junction dynamics in the testis are regulated by an interplay of β1-integrin and focal adhesion complex (FAC)-associated proteins, Endocrinology 144 (2003) 2141–2163.
[140] A. Beardsley, D.M. Robertson, L. O'Donnell, A complex containing α6β1-integrin and phosphorylated focal adhesion kinase between Sertoli cells and elongated spermatids during spermatid release from the seminiferous epithelium, J. Endocrinol. 190 (2006) 759–770.
[141] L. O'Donnell, P.K. Nicholls, M.K. O'Bryan, R.I. McLachlan, P.G. Stanton, Spermiation: the process of sperm release, Spermatogenesis 1 (2011) 14–35.
[142] M.K.Y. Siu, C.H. Wong, W. Xia, D.D. Mruk, W.M. Lee, C.Y. Cheng, The β1-integrin-p-FAK-p130Cas-DOCK180-RhoA-vinculin is a novel regulatory protein complex at the apical ectoplasmic specialization in adult rat testes, Spermatogenesis 1 (2011) 73–86.
[143] M.K.Y. Siu, C.H. Wong, W.M. Lee, C.Y. Cheng, Sertoli-germ cell anchoring junction dynamics in the testis are regulated by an interplay of lipid and protein kinases, J. Biol. Chem. 280 (2005) 25029–25047.
[144] M. Dym, Basement membrane regulation of Sertoli cells, Endocr. Rev. 15 (1994) 102–115.
[145] L. Su, D.D. Mruk, P.P.Y. Lie, B. Silvestrini, C.Y. Cheng, A peptide derived from laminin-γ3 reversibly impairs spermatogenesis in rats, Nat. Commun. 3 (2012) 1185, http://dx.doi.org/10.1038/ncomms2171.
[146] H.H.N. Yan, D.D. Mruk, E.W.P. Wong, W.M. Lee, C.Y. Cheng, An autocrine axis in the testis that coordinates spermiation and blood–testis barrier restructuring during spermatogenesis, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 8950–8955.
[147] Y. Gao, D.D. Mruk, W.Y. Lui, W.M. Lee, C.Y. Cheng, F5-peptide induces aspermatogenesis by disrupting organization of actin- and microtubule-based cytoskeletons in the testis, Oncotarget 7 (2016) 64203–64220.
[148] A. Pozzi, P.D. Yurchenco, R.V. Iozzo, The nature and biology of basement membranes, Matrix Biol. 57–58 (2017) 1–11.
[149] P.D. Yurchenco, Integrating activities of laminins that drive basement membrane assembly and function, Curr. Top. Membr. 76 (2015) 1–30.
[150] H.H.N. Yan, D.D. Mruk, C.Y. Cheng, Junction restructuring and spermatogenesis: the biology, regulation, and implication in male contraceptive development, Curr. Top. Dev. Biol. 80 (2008) 57–92.
[151] B. Shen, M.K. Delaney, X. Du, Inside-out, outside-in, and inside-outside-in: G protein signaling in integrin-mediated cell adhesion, spreading, and retraction, Curr. Opin. Cell Biol. 24 (2012) 600–606.
[152] P. Hu, B.H. Luo, Integrin bi-directional signaling across the plasam membrane, J. Cell. Physiol. 228 (2013) 306–312.

[153] G. Fu, W. Wang, B.H. Luo, Overview: structural biology of integrins, Methods Mol. Biol. 757 (2012) 81–99.
[154] X. Xiao, D.D. Mruk, W.M. Lee, C.Y. Cheng, c-Yes regulates cell adhesion at the blood–testis barrier and the apical ectoplasmic specialization in the seminiferous epithelium of rat testes, Int. J. Biochem. Cell Biol. 43 (2011) 651–665.
[155] X. Xiao, D.D. Mruk, C.Y. Cheng, c-Yes regulates cell adhesion at the apical ectoplasmic specialization-blood–testis barrier axis via its effects on protein recruitment and distribution, Am. J. Physiol. Endocrinol. Metab. 304 (2013) E145–E159.
[156] X. Xiao, D.D. Mruk, E.W.P. Wong, W.M. Lee, D. Han, C.K.C. Wong, C.Y. Cheng, 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 (2014) E553–E562.
[157] Y. Gao, D. Mruk, H. Chen, W.Y. Lui, W.M. Lee, C.Y. Cheng, Regulation of the blood–testis barrier by a local axis in the testis: role of laminin alpha2 in the basement membrane, FASEB J. 31 (2017) 584–597.
[158] Y. Gao, H. Chen, W.Y. Lui, W.M. Lee, C.Y. Cheng, Basement membrane laminin α2 regulation of BTB dynamics via its effects on F-actin and microtubule (MT) cytoskeletons is mediated through mTORC1 signaling, Endocrinology 158 (2017) 963–978.
[159] L. Su, I.A. Kopera-Sobota, B. Bilinska, C.Y. Cheng, D.D. Mruk, Germ cells contribute to the function of the Sertoli cell barrier, Spermatogenesis 3 (2013) e26460.
[160] M. Quiros, A. Nusrat, RhoGTPases, actomyosin, sginaling and regulation of the epithelial apical junctional complex, Semin. Cell Dev. Biol. 36 (2014) 194–203.
[161] T. Suzuki, Regulation of interstinal epithelial permeability by tight junctions, Cell. Mol. Life Sci. 70 (2013) 631–659.
[162] Y. Wang, H. Chang, A. Rattner, J. Nathans, Frizzled receptors in development and disease, Curr. Top. Dev. Biol. 117 (2016) 113–139.