Focal adhesion kinase is a regulator of F-actin dynamics
New insights from studies in the testis

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Introduction

Spermatogenesis takes place in the seminiferous tubule—the functional unit in the testis—to produce spermatozoa (haploid, 1n) from spermatogonia (diploid, 2n) via spermatogenesis.1-3 This highly complex cellular process contains four distinct events, namely mitosis, meiosis, spermiogenesis, and spermiation that constitute the seminiferous epithelial cycle. The seminiferous epithelium, on the other hand, is anatomically segregated into two compartments, the basal and the apical compartment, by the blood-testis barrier (BTB) (Fig. 1). The BTB is constituted by multiple co-existing junctions: (1) testis-specific adherens junction (AJ) called basal ectoplasmic specialization (basal ES), (2) tight junction (TJ), and (3) gap junction (GJ), which together with (4) desmosome at the Sertoli cell-cell interface near the basement membrane of the tunica propria, create one of the tightest blood-tissue barriers in mammals (Fig. 1).4-6 Thus, in the mammalian testis, endothelial TJ barrier of the microvessels in the interstitium contributes virtually no barrier function of the BTB.4,7 The hallmark ultrastructural feature of the BTB, unlike all other blood-tissue barriers,8 is the tightly packed actin filament bundles that line perpendicular to the Sertoli cell plasma membrane, which are sandwiched in-between cisternae of endoplasmic reticulum and the apposing Sertoli cell plasma membranes.9 This unusual ultrastructural feature of the BTB was first described in the early 1970s in the testis.10,11 The term ectoplasmic specialization (ES) was subsequently used in the late 1970s when similar bundles of actin filaments were also found at the Sertoli – spermatid and Sertoli cell-cell interface and designated apical and basal ES, respectively.12-15 The only ultrastructural difference between the apical and basal ES is that the actin filament bundles are not found in the spermatid (step 8-19 spermatids) but restricted only to the Sertoli cell, such that there are only a single array of actin filament bundles at the apical ES vs. two layers of these F-actin bundles at the basal ES (Fig. 1). It is also these actin filament bundles that confer the unusual adhesive strength to the ES in the testis,16,17 making the BTB one of the tightest blood-tissue barriers.8 However, the basal ES/BTB undergoes extensive restructuring at stage VIII of the epithelial cycle to facilitate the transit of preleptotene spermatocytes across the BTB to enter the adluminal compartment to prepare for meiosis.18,19 Furthermore, the apical ES also restructures extensively during spermiogenesis to facilitate the transport of spermatids across the seminiferous epithelium during the epithelial cycle. Once the apical ES forms at the Sertoli-step 8 spermatid interface, it is the only anchorage device, replacing desmosome and GJ, and persists until step 19 spermatids that line up at the luminal edge of the seminiferous tubule to prepare for the release of sperm at spermiation.19,21 Thus, it is conceivable that during spermatogenesis, extensive junction restructuring and cytoskeletal reorganization take place in the seminiferous epithelium to facilitate the transport of: (1)
Spermatogenesis Volume 3 Issue 3

preleptotene spermatocytes across the BTB and (2) spermatids across the epithelium during the epithelial cycle, yet the regulatory biomolecules and/or mechanism(s) remain elusive until recently.

Focal adhesion kinase (FAK), a non-receptor protein tyrosine kinase, was first shown in the late 1980s to be highly expressed in the testis with an expression level significantly higher than that of other non-gonadal tissues. Although this finding suggested its physiological significance in regulating cellular event in testes, its function has remained unexplored until almost 15 y later when FAK was first reported to be a crucial regulator of junction dynamics in spermatogenesis. Herein, we summarize and critically evaluate the recent findings on the role of FAK in the testis.

Focal adhesion kinase (FAK). Focal adhesion kinase (FAK, ~120 kDa), also called protein tyrosine kinase 2 (PTK2), is a non-receptor protein tyrosine kinase, which has been found to be expressed ubiquitously in mammalian tissues including brain, lymphocytes, and testes. FAK contains four linearly arranged functional domains from its N terminus: the band 4.1, Ezrin, Radixin, Moesin (FERM) domain, the catalytic kinase domain, three proline-rich regions, and the focal adhesion targeting (FAT) domain. FAK, as its name implies, is restricted to the focal contact (or focal adhesion complex, FAC), which is an actin-based anchoring junction limited to the cell-extracellular matrix (ECM) interface in mammalian tissues. It is mostly used by motile cells such as fibroblasts, lymphocytes, and metastatic cancer cells for their movement over basal lamina under physiological (e.g., growth, inflammation, combat bacterial/viral infection) or pathophysiological conditions (e.g., tumorigenesis). In the testis, however, FAC (or focal contact) is absent in the seminiferous tubules at the Sertoli cell-basement membrane (BM) interface since the BM is a modified form of ECM in the testis. Instead, FAK is found at the Sertoli cell-cell interface at spermatogenesis.
the basal ES in the BTB and also at the Sertoli-spermatid interface at the apical ES.\textsuperscript{33,32,33} FAK, besides being a kinase that phosphorylates downstream signaling target proteins, also functions as a scaffolding and adaptor protein that mediates the assembly of signaling protein complexes via its protein-protein-interacting domains along its polypeptide sequence, in particular, to transducing the integrin-based signals.\textsuperscript{34-36} It plays an important role in regulating cell proliferation, apoptosis, and cell motility.\textsuperscript{36,37} An elevated expression of FAK also correlates with tumor cell proliferation and metastasis.\textsuperscript{38-40} In fact, FAK is an oncogene and a therapeutic target of cancer therapy.\textsuperscript{41,42} Collectively, these findings illustrate the pivotal role of FAK in cellular functions, both in health and in disease.

In order to exert its intrinsic kinase activity, FAK must first be activated. There are six putative tyrosine phosphorylation sites in FAK, including Tyr-397, -407, -576, -577, -861, and -925,\textsuperscript{35,43} and among them, Tyr-397 is the only autophosphorylation site. Upon phosphorylation of Tyr-397, a high-affinity binding site for Src homology 2 (SH2) domain is exposed that allows FAK to act as an adaptor protein to assemble various SH2 domain-containing regulatory proteins, such as Src family kinases.\textsuperscript{34,44,45} to assemble a multiprotein functional complex. While Tyr-397 is autophosphorylated, other Tyr residues in FAK are phosphorylated by Src family kinases and, in turn, lead to respective downstream effects, illustrating the tight physiological relationship between FAK and Src kinases. In fact, the FAK-Src dual kinase complex is an emerging target in cancer therapy,\textsuperscript{46} and a crucial functional protein complex in cellular physiological events. Apart from tyrosine phosphorylation, FAK can also be phosphorylated on several Ser residues. For instance, Ser-722 phosphorylation inhibits the intrinsic FAK kinase catalytic activity,\textsuperscript{47} while Ser-732 phosphorylation leads to changes in microtubule organization, nuclear movement, and neuronal migration.\textsuperscript{48}

**FAK is a regulator of the apical ES.** Apical ES is an F-actin-rich cell-cell AJ restricted to the Sertoli-spermatid (step 8–19 and 8–16 spermatids in the rat and mouse testis, respectively) interface. FAK was first identified in the rat testis by fluorescence microscopy and shown to be a component in the basal compartment of the seminiferous epithelium as well as at the apical ES.\textsuperscript{27} Further studies have shown that the activated forms of FAK, phosphorylated (p)-FAK-Tyr\textsuperscript{397} and also p-FAK-Tyr\textsuperscript{576}, are restricted to the apical ES and display stage-specific and spatiotemporal expression at the apical ES at stage VI–VIII of the epithelial cycle, whereas FAK is most predominant at the BTB in virtually all stages of the cycle.\textsuperscript{25} Furthermore, p-FAK-Tyr\textsuperscript{397} forms a complex with the apical ES-associated proteins such as β1-integrin, c-Src, and vinculin complex,\textsuperscript{23} indicating its role in mediating β1-integrin signaling pathway at the apical ES. Subsequent studies have confirmed that p-FAK-Tyr\textsuperscript{397} is an integrated component of the α6β1-integrin-based adhesion complex at the apical ES, which persists until spermiation.\textsuperscript{32} Thus, p-FAK-Tyr\textsuperscript{397} is likely a crucial protein in conferring spermatid adhesion and also a regulator during the release of sperm at spermiation.\textsuperscript{70} Furthermore, FAK that works in concert with its partner proteins can create a giant regulatory protein complex composed of p130Cas (p130 Crk-associated substrate), DOCK180 (Dedicator of cytokine 180), RhoA and vinculin (and its associated partners such as Crk, R-ras, and Grb2), which, in turn, is associated with β1-integrin.\textsuperscript{49} In studies using Sertoli-germ cell co-cultures and rats treated with adjudin (a contraceptive drug known to induce apical ES and other anchoring junction restructuring in the testis)\textsuperscript{4} to investigate spermatid adhesion, the β1-integrin-p-FAK-p130Cas-DOCK180-RhoA-vinculin complex emerges as a crucial role in mediating alterations on the actin-based cytoskeleton and subsequently modulating spermatid transport and spermiation during spermatogenesis.

With the discoveries of the structural components (e.g., integral membrane protein β1-integrin, adaptor proteins vinculin, and paxillin) that are associated with FAK at the apical ES, in particular, its three activated forms p-FAK-Tyr\textsuperscript{397}, p-FAK-Tyr\textsuperscript{576}, and p-FAK-Tyr\textsuperscript{407}, and their unique stage-specific and spatiotemporal expression in the seminiferous epithelium,\textsuperscript{23,33} these observations implicate their likely roles in regulating apical ES dynamics during spermatid transport and spermiation via a modulation of actin filament network in the seminiferous epithelium. For instance, while p-FAK-Tyr\textsuperscript{397} and -Tyr\textsuperscript{407} are both highly expressed at the apical ES at stage VII of the epithelial cycle, p-FAK-Tyr\textsuperscript{397} is restricted to the convex side of the spermatid head and co-localized with β1-integrin,\textsuperscript{23,33} whereas p-FAK-Tyr\textsuperscript{407} is expressed almost exclusively to the concave side of the spermatid head and co-localized with Arp3.\textsuperscript{33} Arp3 (actin-related protein 3, which together with Arp2 forms the Arp2/3 complex, which can be activated by N-WASP, neuronal Wiskott-Aldrich syndrome protein\textsuperscript{51,52}) is known to induce barbed end nucleation of an existing actin filament, thus effectively creating an extensive branched actin network. In short, the N-WASP/Arp2/3 protein complex effectively converts actin filaments from a “bundled” to a “de-bundled/branched” configuration, thereby destabilizing the ES-based cell adhesion and to facilitate endocytic vesicle-mediated protein trafficking.\textsuperscript{31,54} Indeed, recent studies have shown that this site of the apical ES at the concave side of the spermatid head is where endocytic vesicle-mediated protein trafficking takes place to facilitate endocytosis, transcytosis, and recycling of apical ES proteins, such that “old” apical ES proteins can be used to assemble “new” apical ES derived from step 8 spermatids via spermiogenesis.\textsuperscript{21,54} On the other hand, the convex side of the spermatid head is being used to confer spermatid adhesion at stage VII of the epithelial cycle since both Eps8 (epidermal growth factor receptor pathway substrate 8, an actin barbed end capping and bundling protein) and palladin (an actin cross-linking and bundling protein) are also highly expressed at this site\textsuperscript{55,56} when p-FAK-Tyr\textsuperscript{397} is upregulated.\textsuperscript{33} These actin bundling proteins can thus be used to maintain the integrity of the actin filament bundles at the convex side of the spermatid head to anchor these spermatids onto the Sertoli cell in the epithelium. Interestingly, at late stage VIII of the epithelial cycle, the expression of p-FAK-Tyr\textsuperscript{397}, Eps8, and palladin; as well as p-FAK-Tyr\textsuperscript{407} and Arp3 are all subsided considerably and they are virtually non-detectable at the apical ES to facilitate the release of sperm at spermiation.\textsuperscript{33,55-57} In short, it is highly likely that these two forms of p-FAK regulate the intrinsic activity of these actin bundling and nucleation proteins to induce re-organization of the network of actin filament bundles at the apical ES during the epithelial cycle to facilitate both spermatid transport across...
the epithelium during spermatogenesis and the release of sperm at spermatogenesis. This conclusion is supported by findings in a recent report, which have demonstrated that overexpression of a p-FAK-Tyr407 phosphomimetic mutant FAK Y407E in Sertoli cells with an established TJ-permeability barrier significantly enhances the kinetics of actin polymerization,33 illustrating that p-FAK-Tyr407 at the apical ES can indeed modify the organization of the F-actin network. Furthermore, the downregulation of c-Yes by -70% in the testis in vivo by RNAi also impedes the localization and downregulates the expression of p-FAK-Tyr407 at the apical ES, causing defects in spermatogenesis in which elongated spermatids are trapped deep inside the seminiferous epithelium in stage VIII tubules, failing to undergo spermiogenesis and these spermatids also display a loss of polarity in which their heads are no longer pointing toward the basement membrane but aligned randomly in the seminiferous epithelium.58 More important, this downregulation of p-FAK-Tyr407 following the knockdown of c-Yes in the testis also associates with changes in actin polymerization.58 Taken collectively, these findings have unequivocally demonstrated that the regulating roles of these phosphorylated FAK forms in F-actin reorganization at the apical ES. Furthermore, several protein kinases (e.g., PKB), lipid kinases (e.g., PI3K), and regulatory proteins (e.g., RhoA GTPase, DOCK180) that are known to be involved in regulatory actin dynamics are also binding partners of FAK and/or its phosphorylated/activated form(s) at the apical ES.4,9,21,62,63 Interestingly, FAK is structurally associated with the occludin-ZO-1 complex at the BTB instead of restricted to the Sertoli cell-basement membrane since FAC is absent in the testis.4 Subsequent studies have shown that occludin is a putative substrate of FAK, since the knockdown of FAK at the Sertoli cell BTB alters the phosphorylation status of occludin, impeding occludin-ZO-1 association, thereby destabilizing the Sertoli cell TJ-permeability barrier.67 These findings thus illustrate the pivotal role of FAK in conferring adhesion function at the Sertoli cell BTB via its effects on the phosphorylation status of the occludin-ZO-1 complex.

A more recent report using various mutants of p-FAK-Tyr97 and -Tyr407 for their overexpression in Sertoli cells cultured in vitro with a functional TJ-permeability barrier that mimics the BTB in vivo has shown that p-FAK-Tyr407 is promoting the Sertoli cell BTB function, tightening the TJ-barrier.33 However, p-FAK-Tyr97 is promoting the BTB disruption, making the Sertoli cell TJ-permeability barrier “leaky.”68 In short, the p-FAK-Tyr407 and -Tyr97 forms of FAK have antagonistic effects on the Sertoli cell BTB, illustrating these two non-receptor protein tyrosine kinases may serve as molecular switches to turn “on” and “off” the TJ-barrier during the transit of preleptotene spermatocytes across the BTB at stage VIII of the epithelial cycle. This concept, besides supported by the antagonistic effects of these two forms of FAK, is also strengthened by the stage-specific and spatiotemporal expression of p-FAK-Tyr407 at the BTB as well as its association with Arp3 of the Arp2/3 protein complex. For instance, p-FAK-Tyr407 is structurally associated with N-WASP,33 suggesting N-WASP is also a substrate of FAK and overexpression of p-FAK-Tyr407 phosphomimetic mutant in the Sertoli cell epithelium that promotes the Sertoli TJ-barrier function also induces an increase in the association of Arp3 and N-WASP.33 These findings are important because they illustrate that FAK exerts its effects via its p-FAK-Tyr407 and -Tyr97 forms

| Regulators | Functions | References |
|------------|-----------|------------|
| PI3K, PKB, RhoA, DOCK180 | Interacts with p-FAK-Tyr97; initiates PI3K/PKB signaling pathway and cross-talks to ERK signaling pathway; facilitates the establishment of AJ in Sertoli-germ cell co-culture | 50 |
| sFRP1 | Acts upstream of p-FAK-Tyr407; members of Wnt signaling pathway that promotes spermatid adhesion at the apical ES | 87 |
| c-Yes | Acts upstream of p-FAK-Tyr407; initiates c-Src signaling pathway and enhances spermatid adhesion | 58, 88 |

AJ, adherens junction; Akt, transforming retrovirus of Ak strain that induces thymoma, spontaneous thymic lymphomas first identified in mouse, also known as protein kinase B (PKB) which is a Ser/Thr-specific protein kinase; DOCK180, dedicator of cytokinesis; ERK, extracellular signal-regulated kinase; PI3K, phosphoinositide 3-kinase; sFRP1, secreted Frizzled-related protein 1; Wnt, Wingless-MMV Integration Site.
to regulate F-actin organization at the BTB by modulating the conversion of actin filaments from a “bundled” to a “de bundled/branched” configuration, conferring plasticity to the F-actin network at the ES. Furthermore, the two phosphorylated forms of FAK are known to interact with several regulatory proteins. For instance, SHP2 (Src homology domain-containing phosphatase-2, a ubiquitously expressed non-receptor protein tyrosine phosphatase in mammalian cells, also known as PTPN11, tyrosine-protein phosphatase non-receptor type 11, an enzyme encoded by PTPN11 gene in humans) is known to downregulate the expression of p-FAK-Tyr397 and initiates the mitogen-activated kinase (MAPK) signaling pathway, subsequently modulating actin cytoskeleton.68

Figure 2 is a schematic drawing which depicts a hypothetical model on the role of FAK in regulating F-actin organization at the BTB during the epithelial cycle of spermatogenesis.

FAK and the apical ES-BTB-BM (apical ectoplasmic specialization-blood-testis barrier-basement membrane) functional axis in the testis. Since the initial discovery of the seminiferous epithelial cycle of spermatogenesis in the 1950–60s in rodents and humans,69-72 it is known that cellular events that occur across the seminiferous epithelium are tightly regulated.18,19,73,74 However, the molecular basis that coordinates these events is virtually unknown until a report published in 2008,75 demonstrating for the first time the presence of a local functional axis that coordinates these events known as the apical ES-BTB-BM axis.21,75 In this first report,75 it was shown that overexpression of fragments of laminin chains (note: laminins, such as laminin-α3β3γ3, are components of the adhesion protein complex at the apical ES76-78) or inclusion of purified recombiant proteins of these fragments in Sertoli cells cultured in vitro with an established TJ-permeability barrier, they both perturbed the Sertoli cell TJ-barrier function. These observations thus suggest that MMP-2 (matrix metalloprotease-2), which is highly expressed at the apical ES at stage VIII of the epithelial cycle,77 likely cleaves laminin chains at the apical ES during its degeneration at spermatiation to generate the biologically active fragments to induce BTB restructuring, thereby coordinating the cellular events of spermatiation and BTB restructuring that take place concurrently but at the opposite ends of the epithelium at stage VIII of the epithelial cycle. In short, there is a functional axis between the apical ES and the BTB, which is mediated by the autocrine-based laminin fragments. Since apical ES was absent in these cultures due to the lack of elongating/elongated spermatids, the knock-down of β1-integrin by RNAi (note: β1-integrin is a component of the apical ES and also the hemidesmosome at the Sertoli cell-BM interface) was also found to induce BTB restructuring.73 Thus, the BTB and the hemidesmosome at the BM are also functionally linked. Additionally, recent studies have shown that biologically active fragments are also released by collagen chains in the BM that regulate BTB function, confirming the presence of the BTB-BM axis.79 This apical ES-BTB-BM functional axis has since been confirmed in which the biologically active domain of at least two laminin chains are identified and they have shown to be potent biologically active peptides to regulate Sertoli BTB function both in vitro and in vivo in a reversible fashion.80 Furthermore, studies using the phthalate-induced Sertoli cell injury model have also confirmed the presence of this local functional axis in the testis.81-83

A recent report has shown that the p-FAK-Tyr397 and p-FAK-Tyr407 are the likely “on” and “off” molecular switches in this apical ES-BTB-BM functional axis that modulate the organization of actin filament bundles at the apical ES, as well as the basal ES. For instance, p-FAK-Tyr407 and p-FAK-Tyr397 promotes and disrupts the Sertoli cell TJ-permeability barrier function,
respectively, which is mediated via their effects on the organization of F-actin network at the BTB. In short, biologically active laminin fragments released from the apical ES can alter the spatiotemporal expression of these molecular “switches” in the seminiferous epithelium, which, in turn, affects re-organization of F-actin at the basal ES, promoting BTB restructuring. This hypothesis is supported by findings that following administration of the biologically active laminin F5 peptide, there is a downregulation and mis-localization of p-FAK-Tyr at the apical and basal ES, which is also associated with a disruption of F-actin organization at both sites, leading to spermatid loss.

from the epithelium and BTB disruption. At present, the receptor(s) for the laminin fragments, such as F5 peptide, at the BTB is unknown, but β1-integrin is the likely receptor of the laminin fragments at the BM. It is likely that the p-FAK-Tyr and -Tyr serve as the downstream regulators of the laminin fragment (ligand)-integrin (receptor) complex in this functional axis that coordinates different cellular events that take place across the seminiferous epithelium during the epithelial cycle. Figure 3 is a schematic drawing that illustrates a hypothetical model, in particular, the early signaling cascades along the apical ES-BTB functional axis in the seminiferous epithelium.

**Concluding Remarks and Future Perspectives**

Herein, we briefly summarize the critical role of FAK in the seminiferous epithelium of the rat testis. It is likely that the stage-specific and spatiotemporal expression of p-FAK-Tyr and p-FAK-Tyr at the apical and/or basal ES serve as the downstream signal transducers of the laminin (ligand)-integrin (receptor) complex in the apical ES-BTB-BM functional axis. These signaling complexes either are working in concert with adhesion protein complexes at the ES (e.g., occludin-ZO-1 complex) or actin regulatory proteins (e.g., the N-WASP-Arp2/3 complex, palladin, drebrin E, Eps8) to modulate cell adhesion function and the organization of F-actin at the ES. A better understanding of FAK in the testis should reveal novel targets for male contraceptive development and also insightful information on toxicant-induced reproductive dysfunction since the apical ES-BTB-BT axis is an emerging target of toxicant-induced male infertility.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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51. Cheng CY, Mruk DD. Regulation of spermiogenesis, spermiation and blood-testis barrier dynamics: novel insights from studies on Eps8 and Arp3. Biochem J 2011; 435:553-62; PMID:21486226; http://dx.doi.org/10.1042/BJ20110321

52. Cheng CY, Mruk DD. Actin binding proteins and spermiogenesis: Some unexpected findings. Spermatogenesis 2011; 1:99-104; PMID:22319657; http://dx.doi.org/10.4161/spmg.1.2.12091

53. Cheng CY, Lie PPY, Wong EWP, Mruk DD, Silvestrini B. Adjudin disrupts spermiogenesis via the action of some unlikely partners: Eps8, Arp2/3 complex, drebrin E, PAR6 and 14-3-3. Spermatogenesis 2011; 1:291-7; PMID:22332112; http://dx.doi.org/10.4161/spmg.1.4.18593

54. Vög AW, Young JS, Du M. New insights into roles of tubulobulbar complexes in sperm release and turnover of blood-testis barrier. Int Rev Cell Mol Biol 2013; 303:319-55; PMID:23445814; http://dx.doi.org/10.1016/B978-0-12-407697-6.00008-8

55. Lie PPY, Mruk DD, Lee WM, Cheng CY. Epidermal growth factor receptor pathway substrate 8 (Eps8) is a novel regulator of cell adhesion and the blood-testis barrier integrity in the seminiferous epithelium. FASEB J 2009; 23:2555-67; PMID:19293393; http://dx.doi.org/10.1096/fj.08-107973

56. Qian X, Mruk DD, Wong EWP, Lie PPY, Cheng CY. Palladin is a regulator of actin filament bundles at the ectoplasmic specialization in adult rat testes. Endocrinology 2013; 154:1907-20; PMID:23546604; http://dx.doi.org/10.1209/endo.2012-2269

57. Lie PPY, Chan AYN, Mruk DD, Lee WM, Cheng CY. Restricted Arp3 expression in the testis prevents blood-testis barrier disruption during junction restructuring at spermatogenesis. Proc Natl Acad Sci USA 2009; 106:9928-303; PMID:19470647; http://dx.doi.org/10.1073/pnas.0813113106

58. Puri P, Walker WH. The tyrosine phosphatase SHP2 regulates Sertoli cell junction complexes. Biol Reprod 2013; 88:59; PMID:23235809; http://dx.doi.org/10.1095/biolreprod.112.104414

59. Leblond CP, Clermont Y. Spermiogenesis of rat, mouse, and hamster and guinea pig as revealed by the periodic acid-silver methenamine method. J Cell Biol 1959; 48:37-56; PMID:13810668

60. Clermont Y. Kinetics of spermatogenesis in mammalian seminiferous epithelium cycle and spermatogonial renewal. Physiol Rev 1972; 52:198-236; PMID:4621362

61. Clermont Y. The cycle of the seminiferous epithelium in man. Am J Anat 1963; 112:35-51; PMID:14021715

62. Campbell M, Humphries P. The blood-retina barrier: tight junctions and barrier modulation. Adv Exp Med Biol 2012; 763:70-84; PMID:22397619

63. Easton AS. Regulation of permeability across the blood-brain barrier. Adv Exp Med Biol 2012; 763:1-19; PMID:22397617

64. Dym M, Fawcett DW. The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. Biol Reprod 1970; 3:308-26; PMID:4180372

65. Cheng CY, Mruk DD. Cell junction dynamics in the testis: Sertoli-germ cell interactions and male contraceptive development. Physiol Rev 2002; 82:825-74; PMID:12270945

66. Siu ER, Wong EW, Mruk DD, Sie KL, Porto CS, Cheng CY. An ocludin-focal adhesion kinase protein complex at the blood-testis barrier: a study using the cadmium model. Endocrinology 2009; 150:3336-44; PMID:19213829; http://dx.doi.org/10.1210/en.2008-1741

67. Siu ER, Wong EW, Mruk DD, Porto CS, Cheng CY. Focal adhesion kinase is a blood-testis barrier regulator. Proc Natl Acad Sci USA 2009; 106:9928-303; PMID:19470647; http://dx.doi.org/10.1073/pnas.0813113106

68. Puri P, Walker WH. The tyrosine phosphatase SHP2 regulates Sertoli cell junction complexes. Biol Reprod 2013; 88:59; PMID:23235809; http://dx.doi.org/10.1095/biolreprod.112.104414

69. Leblond CP, Clermont Y. Spermiogenesis of rat, mouse, and hamster and guinea pig as revealed by the periodic acid-silver methenamine technique. Am J Anat 1959; 101:90-167-215; PMID:14923625

70. Clermont Y. Kinetics of spermatogenesis in mammalian seminiferous epithelium cycle and spermatogonial renewal. Physiol Rev 1972; 52:198-236; PMID:4621362

71. Clermont Y. The cycle of the seminiferous epithelium in man. Am J Anat 1963; 112:35-51; PMID:14021715

72. Leblond CP, Clermont Y. Spermiogenesis of rat, mouse, and hamster and guinea pig as revealed by the periodic acid-silver methenamine technique. J Cell Biol 1959; 48:37-56; PMID:13810668

73. Parvinen M. Regulation of the seminiferous epithelium. Am J Reprod Immunol 2006; 55; PMID:21256972; http://dx.doi.org/10.1111/j.1600-089X.2005.00771.x

74. Siu MKY, Cheng CY. Interactions of proteases, protease inhibitors, and the β3 chains that serves as the ligand for α3β1-integrin at the apical ectoplasmic specialization in adult rat testes. Int J Biochem Cell Biol 2011; 43:651-65; PMID:21256972; http://dx.doi.org/10.1016/j.biocel.2011.01.008

75. Siu MKY, Cheng CY. Interactions of proteases, protease inhibitors, and the β3 integrin/laminin γ3 protein complex in the regulation of ectoplasmic specialization dynamics in the rat testis. Biol Reprod 2004; 70:945-64; PMID:14645107; http://dx.doi.org/10.1095/biolreprod.103.026506

76. Koch M, Olof PF, Albus A, Jin W, Hunter DD, Brunken WJ, et al. Characterization and expression of the laminin γ3 chain: a novel, non-basement membrane-associated, laminin chain. J Cell Biol 1999; 145:605-18; PMID:10225960; http://dx.doi.org/10.1083/jcb.145.3.605

77. Siu MKY, Cheng CY. Restricted Arp3 expression in the testis prevents blood-testis barrier disruption during junction restructuring at spermatogenesis. Proc Natl Acad Sci USA 2009; 106:9928-303; PMID:19470647; http://dx.doi.org/10.1073/pnas.0813113106