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The Sertoli Cell and Blood-Testis Barrier

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ABSTRACT The Sertoli cell is a critical somatic cell that initiates the development of testicular morphology and determines important parameters for spermatogenic function. The blood-testis barrier, also known as the Sertoli cell barrier and one of the tightest tissue barriers in the mammalian body, is an immunological barrier to separate post meiotic germ cell antigens from the systemic circulation. Additionally, creating a unique microenvironment for the development of spermatocytes that exceed into the adluminal compartment from the leptotene stage. It restricts the passage of substances such as paracrine factors, electrolytes, hormones, water, and biological molecules to the apical part of the seminiferous tubule. It separates spermatogenic cells from toxic and drug-containing environmentally harmful substances, hormones, and biomolecules in the systemic circulation. This nearly impenetrable barrier prevents proteins, including antibodies, from reaching the spermatogenic cells. It also prevents protein leakage from developing spermatogenic cells and forming an immune response. This review explains Sertoli's functional properties, the testis barrier's molecular structure, the substances involved in the barrier dynamics, and their importance in realizing spermatogenesis.

Keywords: *Blood-testis barrier, Sertoli cell, Testis.*

ÖZ

Sertoli Hücresi ve Kan-Testis Bariyeri

Sertoli hücresi, testis morfolojisinin gelişimini başlatan ve spermatogenik fonksiyon için önemli parametreleri belirleyen kritik bir somatik hücredir. Sertoli hücre bariyeri olarak da bilinen ve memeli vücudundaki en sıkı doku bariyerlerinden biri olan kan testis bariyeri, postmayotik eşey hücre antijenlerini sistemik dolaşımdan ayırmak için immünolojik bir bariyerdir. Ayrıca, leptoten aşamasından adluminal bölmeye geçen spermatositlerin gelişimi için özel bir mikro ortam oluşturur. Su, elektrolitler, iyonlar, hormonlar, parakrin faktörler ve biyolojik moleküller gibi bazı maddelerin Sertoli hücrelerinin arasından apikale doğru geçişini kısıtlar. Spermatogenik hücreleri toksik ve ilaç içeren çevresel zararlı maddelerden, sistemik sirkulasyondaki hormonlar ve biyomoleküllerden ayırır. Neredeyse geçirimsiz olan bu bariyer, antikorlar da dahil olmak üzere proteinlerin spermatogenik hücrelere ulaşmasını engeller. Tersi yönde de gelişmekte olan spermatogenik hücrelerden protein sızmasını ve immun cevabı tetiklemesini engeller. Bu derlemede, Sertoli hücrelerinin fonksiyonel özellikleri ile kan-testis bariyerinin moleküler yapısı, bariyer dinamiklerine dahil olan maddeler ve spermatogenezisin gerçekleşmesindeki önemi ile ilgili bilgiler verilmesi amaçlanmıştır.

Anahtar Kelimeler: *Kan-testis bariyeri, Sertoli hücresi, Testis.*

INTRODUCTION

Sertoli cells, one of the somatic cells of the seminiferous tubules, are cells that start from the basal membrane of the tubule and extend to its lumen, and have numerous apical and lateral extensions. With these extensions, they surround the spermatogenic cells between them. Due to their close contact, they are supportive and nutritive cells for developing germ cells. These cells, which lose their ability to divide when fully specialized, do not reproduce after puberty (Skinner and Griswold 2005).

It has been reported that the formation of the blood barrier in the testis is fully formed in the postnatal period (Mok et al. 2011). The epithelium of the seminiferous is divided into two compartments by this barrier. One is the basal compartment with spermatogonium, and leptotene spermatocytes, the other one is the apical (adluminal) compartment the place of primary and secondary spermatocytes and spermatids are observed. Leptotene spermatocytes must cross this barrier to enter the adluminal compartment. The testis barrier is a structure that goes through “opening” and “closing” cycles to provide this germ cell migration (Skinner and Griswold 2005; Wong and Cheng 2005).

This barrier consists of four different cell connections.

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These are tight junctions, desmosomes, ectoplasmic specializations, and gap junctions. Tight junctions are an important component of the barrier. It coexists and cofunctions with desmosomes, ectoplasmic specializations, and gap junctions to constitute a specific environment for completing meiosis and the subsequent spermiogenesis (Wang et al. 2022; Zhixiang et al. 2022). Ectoplasmic specializations, tight junctions, and gap junctions connect to actin microfilaments (Young et al. 2009; Mao et al. 2020), while desmosomes are associated with intermediate filaments (Delva et al. 2009; Lie et al. 2011).

Ectoplasmic specializations are a modified kind of adhesion junction observed solely in the testis. These occur in two major regions in the cell. Two types of ectoplasmic specialization have been described, apical and basal. In the basal region, they occur together with other types of junctions (tight junction, gap junction, and desmosome) to structure massive girdle-like junctional complexes between neighboring Sertoli cells (Wu et al. 2021). Unlike the adhesion belts found in other epithelia, here, on the plasma membrane of two adjacent cells, on the cytoplasmic side of the Sertoli cell, there are bundles of actin filaments sandwiched between the flattened, thinned agranular endoplasmic reticulum vesicles that run parallel along the Sertoli cell membrane and adhesion belt (Siu et al. 2003).

Ectoplasmic specializations contain many proteins such as vinculin, afadin, α-actinin, fimbrin, Eps8 (epidermal growth factor receptor pathway substrate eight), cortactin, espin, myosin VIIa, paxillin, palladin, zyxin, testin, and nectin in addition to actin filaments (Qian et al. 2013; Wen et al. 2018). In addition, several kinases (Wong et al. 2005; Lie et al. 2012) and phosphatases (Puri and Walker 2013) have been localized to regions that regulate filaments and junctions. Nectin-2, nectin-3, zyxin, n-cadherin, e-cadherin, axin, and other proteins are found in the apical ectoplasmic specialization. Ectoplasmic specializations in the basal region, along with other components of the junctional complex, are disrupted as cells migrate adluminal compartments of the epithelium. They are involved in barrier disruption and reorganization during the migration of leptotene spermatocytes into the adluminal compartment. Those in the apical region are disrupted as part of the sperm release mechanism, and new ectoplasmic specializations emerge deeper in the epithelium (Siu et al. 2003; Siu and Cheng 2004).

Structures formed between neighboring Sertoli cells and between Sertoli cells and the head of the spermatid form tubulobulbar complexes. It is a specialized adhesion-attachment complex unique to the testis. They are unique plasma membrane specializations (Young et al. 2009; Traweger et al. 2013). It helps germ cells adhere to Sertoli cell, removes excess spermatid cytoplasm, and eliminates ectoplasmic specialization so that it can remodel the Sertoli barrier before spermiation. Between 4 and 24 tubulobulbar complexes can be found in mature sperm. This indicates that it is important in germ cell movement (Magnanti et al. 2001; Siu et al. 2003). The molecular components of tubulobulbar complexes include elements such as Arp2/3 (actin-related protein), NWASP (neural wiskott-aldrich syndrome protein) (Young et al. 2009), paxillin, cofilin (Duo et al. 2013), cortactin (Young et al. 2009), Eps8, and espin. In addition, amphiphysin, dynamin 2, and dynamin 3 (Vaid et al. 2007) and focal adhesion proteins zyxin and vinculin are also present (Young et al. 2009).

The testis barrier prevents the mixture of molecules between the two compartments. This is an essential property of the Sertoli cell. Because these cells secrete several products like androgen-binding protein (ABP) and test in a polarized manner (Skinner and Griswold 2005). ABP specifically binds testosterone (T) and dihydrotestosterone (DHT). It provides to concentrate in the lumen of the tubules. Thus, the continuity of spermatogenesis is ensured. When the blood-testis barrier is dysfunctional, germ cell differentiation and development cease (Skinner and Griswold 2005; Von Engelhardt et al. 2020).

Differentiation of spermatogonium enables the formation of sperm-specific proteins. Since sexual maturity is reached after the development of the immune system, the differentiated sperm cells may be perceived as foreign and lead to an immune response that can damage the germ cells, the formation of anti-sperm antibodies (ASAs), and secondary infertility. The blood-testis barrier blocks any interaction between the developing sperm and the immune system. This barrier prevents the formation of an antibody against spermatogonium in the immune system, in other words, an autoimmune response, by cutting off the contact of spermatogonium, which are highly antigenic, with blood (Wong and Cheng 2005; Luca et al. 2018).

Molecular Structure of the Blood-Testis Barrier

Both tight junction and adhesion junction in the seminiferous epithelium consist of adaptors, signaling molecules, and integral membrane proteins. Integral membrane proteins in tight junctions; tricellulin, occludins, junctional adhesion molecules (JAM), and claudins (Wen et al. 2018).

Occludin is a 60-65 kDa intercellular adhesion molecule. They are involved in controlling the localization of proteins necessary for cell polarization. Therefore, it is thought to be involved in the arrangement of the movement of leptotene spermatocytes across the blood-testis barrier. (Lui et al. 2003a).

Claudins are another tight junction membrane protein and are thought to provide stronger adhesion than occludins (Lui et al. 2003a). In mammals, the claudin family comprises 24 members. There are seven different claudin molecules in the testis. These are claudin-1, -3, -4, -5, -7, -8, -11. Claudin-11 is expressed in both germ cells and the barrier. It plays an essential role in the integrity of the blood-testis barrier and the process of spermatogenesis. It is very important in terms of the microenvironment that must be created for spermatogenesis. Claudin 5 is expressed by cells of Sertoli, spermatogonia, and leptotene spermatocytes (Morrow et al. 2009).

Three different JAM molecules, named JAM-1, JAM-2, and JAM-3, have been identified. JAMs show a different molecular structure from occludin and claudins. Cadherins, integrins, and nectins are integral membrane proteins existing in the ectoplasmic specialization region (Takai and Nakanishi 2003). Desmosomes contain desmoglein-2 and desmocollin-2 as integral membrane proteins. Recent research has also proven that desmosomes in the blood-testis barrier structure regulate the functions of other junctional proteins such as N-cadherin and occludin in regions where basal ectoplasmic specializations and tight junctions are observed (Lie et al. 2011; Mruk and Cheng 2011a).

Gap junctions are an actin-based type of junction responsible for cell communication with each other. They

are formed as a result of the fusion of channels called conexon. Integral membrane proteins formed these channels called connexins. Connexin 43 and connexin 46 are the most studied gap junction proteins in the testis (Lie et al. 2011; Mruk and Cheng 2011a; Mruk and Cheng 2011b; Mruk and Cheng 2011c).

The communication of these proteins, which are attached to the cytoskeleton via different adapters, is regulated by signaling molecules such as kinases and phosphatases (Wong and Cheng 2005).

Adaptors are significant regulatory molecules in testicular junction dynamics. However, it is not possible to definitively define this regulation mechanism. The binding interest to different structural and signaling proteins at the junction sites will likely cause different results. Adaptors share an important function in remodeling the barrier with different proteins such as kinases and phosphatases (Wong et al. 2004; Wong and Cheng 2005; Wong et al. 2005).

Occludin, claudin, and JAM bind to the cytoskeleton via different adaptors such as afadin, zonula occludens (ZO)-1, ZO-2, and ZO-3. In addition, integral membrane proteins such as nectins, cadherins, and integrins are also associated with cytoskeletal networks through various adapters such as afadin, catenins, vinculin, cortactin, and actin (Wong et al. 2004; Wong et al. 2005).

ZO proteins belong to the membrane-associated guanylate kinase (MAGUK) family, which consists of 10 different subfamilies. These proteins also function outside the tight junctions, such as regulating cell growth and proliferation (Spadaro et al. 2012; Traweger et al. 2013; Hervé et al. 2014).

Regulation of Blood-Testis Barrier Dynamics

The blood-testicular barrier must be physically dissolved to allow the passage of leptotene spermatocytes into the adluminal compartment during spermatogenesis. In this process, there are changes in the expression of structural, binding, and signaling proteins, localization, and modifications in the communication of proteins with each other (Wang et al. 2022).

Research in rats has demonstrated that nitric oxide synthase is also a significant regulator of tight junction dynamics of Sertoli cells (Skinner and Griswold 2005).

Sertoli-germ cell coactions also regulate blood-testis barrier dynamics, sending signals to Sertoli cells to ease germ-cell migration (Wong and Cheng 2005).

Roles of Cytokines in Regulating Blood-Testis Barrier Dynamics

The blood-testis barrier restructuring to facilitate germ cell transport is arranged by cytokines like tumor necrosis factor-α and transforming growth factor-β3 (Lui et al. 2003b).

Germ and Sertoli cells secrete cytokines. They play multiple functions in spermatogenesis, such as regulating cell division, differentiation and ensuring cell survival. In addition, cytokines in the seminiferous epithelium have been shown to be crucial for regulating barrier dynamics through their influences on levels of tight junction and adhesion junction, proteases, protease inhibitors, integral membrane proteins, and extracellular matrix proteins. The most studied cytokines known to be involved in testis so far are tumor necrosis factor α (TNF-α) and transforming growth factor-β3 (TGF-β3) (Lui et al. 2003b, Siu et al. 2003).

TNF-α is a cytokine secreted by cells of Sertoli and germ cells; receptors are limited to Sertoli cells. During the in vitro montage of the blood-testis barrier, the amount of TNF-α produced by Sertoli cells was considerably reduced, suggesting that it can regulate barrier function. Although the mechanism by which TNF-α affects the blood-testis barrier in vivo is not known, proteases and protease inhibitors in the seminiferous epithelium have been shown to modulate barrier integrity by affecting homeostasis (Siu et al. 2003).

TGF-β3 exerts similar regulatory effects on the Sertoli cell tight junction barrier with different signaling pathways. TGF-β3 has the highest expression at the onset of puberty in rats. This is because the Sertoli cells produce it. In addition, spermatogonia and early spermatocytes in adult pig and rat testis also secrete this factor (Lui et al. 2003b).

TGF-β3 receptors are predominantly found in Sertoli cells. Studies show that TGF-β3 arranges the testis barrier dynamics in vivo via the p38 MAPK path and modulates the levels of tight junction and adhesion junction-related proteins (Lui et al. 2003b). These observations were confirmed in vivo using rats treated with CdCl2 in vitro as a model (Chen et al. 2018). For example, during CdCl2-induced barrier disruption, a decrease in occludin and ZO-1 levels in the blood-testis barrier region has been shown, whereas TGF-β3 was considerably induced in the testis (Wong et al. 2004). It is important to expand studies on the roles of these two cytokines in the seminiferous epithelium in the future.

Interaction of Proteases and Protease Inhibitors

Proteases are important for the tissue to regain its shape during spermatogenesis. There are a variety of proteases and protease inhibitors in the testis. They are important in the restructuring event, which includes the timely "opening" and "closing" of the blood-testis barrier during spermatogenesis. Proteases can either be directly related to eliminating junctional constituents during the passage of leptotene spermatocytes into the adluminal compartment; or indirectly by activating other molecules such as extracellular matrix components (i.e., collagen) and growth factors (Wong and Cheng 2005).

There are researches displaying that protease and protease inhibitors regulate blood-testis barrier dynamics. These include α2-macroglobulin (α2-MG, a non-specific protease inhibitor), protein C inhibitor (PCI), gelatinases (metalloproteinases), cathepsins, plasminogen activators (PAs), cystatin C (a cysteine protease inhibitor), and tissue inhibitors of metalloproteinases (TIMPs) (Wong and Cheng 2005).

Sertoli Cell Secreted Regulatory Factors

The regulatory factors are crucial for testis development, spermatogenesis, and the control of male fertility. They are factors that affect cellular function and differentiation on a molecular level. For example, while they affect Sertoli cells autocrine, they affect spermatogenic cells, Leydig, and peritubular myoid cells paracrinely. These factors are hormones and growth factors (Skinner and Griswold 2005).

Growth factors are factors that affect the cell cycle and also have effects on cellular functions and cell differentiation (Skinner and Griswold 2005). Sertoli cell-secreted growth factors such as stem cell factor (kit ligand), insulin-like growth factors (IGF-1 and 2), fibroblast growth factor (FGF), glial cell-derived neurotrophic factor, transforming growth factor alpha and beta, neurotropins, and bone morphogenetic protein

4 (Lui et al. 2003b; Skinner and Griswold 2005; Young et al. 2009; Young and Vogl 2012; Parekh et al. 2019; Hohmann and McBeath, 2022). Sertoli cells are endocrine cells that secrete hormones such as estrogen, activin, inhibin, and antimullerian hormone (Marchetti et al. 2003; Siu et al. 2003; Traweger et al. 2013; Von Engelhardt et al. 2020). Sertoli cells also produce ciliary neurotrophic factor (CNTF), erythropoietin, and leukemia inhibitory factor (LIF). First, however, these factors' specific expressions and effects must be evaluated (Magnanti et al. 2001).

In conclusion, Sertoli cell and blood-testicular barrier are critical in spermatogenesis. Infertility may occur if this barrier's structure and function are impaired. We think that this review, which examines the structure of the barrier, which provides an optimal environment by protecting the germ cells against both immunological and environmental effects, may also be a reference for studies on the biology of the barrier and male reproduction.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: YA, EE
Supervision / Consultancy: EE
Writing the Article: YA

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