Molecular Architecture and Functional Dynamics of Blood Testis Barrier

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Introduction

Tight junctions (TJs) are one mode of cell-to-cell adhesion, and play a central role in sealing the intercellular space in epithelial and endothelial cellular sheets, and in creating and establishing apical and basolateral membrane domains in these types of cells. Through these "barrier" and "fence" functions of TJs, epithelial/endothelial cellular sheets establish various compositionally distinct fluid compartments. TJ is a multimolecular membrane specialization comprising multiple integral membrane proteins, occludin, claudins and junctional adhesion moleculae (JAM), and several associated peripheral proteins. The latter forms a cytoplasmic plaque which interacts with actin filaments. In testis, TJ between Sertoli cells is an important structural element of the blood testis barrier (BTB) which creates a regulated paracellular barrier to the movement of water, solutes, and immune cells from circulation to seminiferous tubule. Functional BTB is formed at the time of the puberty and crucial for spermatogenesis. Pathologic condition in BTB is related with diverse fertility status in men. Although recent studies have focused on the cloning and tissue distribution of the genes which build up TJs, molecular architecture of TJ and the functional dynamics of inter-Sertoli TJs and thus BTB are still far from understanding.

Structure and functional aspects of BTB

Genes build up inter-Sertoli TJs: To date, expression of several TJ genes

are found in testis. Of these, occludin, claudin-1, 11, and JAM is expressed in Sertoli cells. Interestingly, expression of these genes is developmentally changed in testis. Claudin-1 and occludin is preferentially expressed in fetal and early postnatal testes. On the other hand, the expression of claudin-11, Sertoli cell-specific integral member of TJ is increased during pubertal development. Reciprocal expression of claudin-1 and claudin-11 during testis development suggests that structure and function of inter-Sertoli TJs and thus BTB are determined by the types of claudins expressed in Sertoli cells. Relative expression of claudin-1 and claudin-11 might be one of the key characters determining the differentiation of seminiferous epithelia according to the progression of spermatogenesis. Recently, TJ strands made by some claudins have been known to show ion selectivity. This is much of concern because this can create different ionic composition between interstitium and lumen of the seminiferous tubule through the paracellula route. It has been long been believed that the difference in ionic composition between interstitium and lumen of the seminiferous tubule is established by ion transport via transcellular pathway in Sertoli cells. However, the types of claudins in inter-Sertoli TJs may be implicated in the establishment of differential ionic composition between interstitium and lumen of the seminiferous tubule during the luminogenesis in seminiferous tubules. JAM not only mediates homotypic cell-cell interactions, it also facilitates the transepithelial migration of neutrophils across TJs. As preleptotene and leptotene spermatocytes reside outside the BTB, these cells must gain entry into the adluminal compartment traversing the BTB during spermatogenesis. As such, the BTB mustdisassemble and reassemble to allow the translocation of germ cells across this barrier for further development at the adluminal compartment. In mouse, early prepubertal (PND15) testis which is largely populated by early spermatocytes shows high level of JAM expression, suggesting specific expression of JAM during the translocation of early spermatocytes from basolateral site to adluminal compartment. Spatio-temporal pattern of JAM expression during postnatal development of testis and at different seminiferous epithelial cycles in adult testis will give us important informations about the remodeling of inter-Sertoli TJ during the

differentiation of early spermatocytes.

Endocrine and paracrine regulation of inter-Sertoli TJs

Transforming growth factor-β (TGF-β): The structure and functions of TJ are under control of paracrine and endocrine as well as physiochemical factors in various tissues. In testis, transforming growth factor-β3 (TGF-β3) down regulates expression of several TJ genes such as ZO-1, occludin, claudin-11 in Sertoli cells via p38 MAPK dependent manner and thus perturbs BTB. This enables the early spermatocytes to translocate from basolateral to adluminal compartment of seminiferous tubule. In seminiferous tubule, TGF-β3 is expressed in meiotic germ cells as well as Sertoli cells, and TGF receptor I (TβRI) and II (TβRII) in germ cells including spermatogonia and spermatocytes as well as Sertoli cells. Smad2, a R-Smad for TGF-β is found together with Smad4 in the seminiferous epithelium and the strongest in pachytene spermatocytes. This may fulfils requirement for functional crosstalk between Sertoli cells and germ cells to remodel the inter-Sertoli TJs through the TGF-β receptor signaling in Sertoli cells. However detail of this event is still largely uncovered.

TNF-α: Spatiotemporally regulated interactions between germ cells and Sertoli cells are crucial for adequate remodeling of BTB during spermatogenesis. TNF-α, a cytokine produced by both Sertoli and germ cells, can perturb Sertoli cell TJ function *in vitro* possibly via its effects in perturbing the homeostasis of proteases and protease inhibitors in the ECM adjacent to the TJ site utilizing the integrin/ILK/SAPK/JNK signalling pathway, which in turn regulate the Sertoli cell TJ function. To date, however, the changes in molecular architecture of TJ are still uncovered.

Steroids: Undoubtedly, testosterone produced by Leydig cell is the most important factor for spermatogenesis. Testosterone increases the expression of TJ genes such as occludin and claudin-11 and potentiates TER inSertoli cells. Glucocorticoid and its synthetic analogue dexametasone stimulate TJ development in several epithelia through the up-regulation of TJ genes expression and subcellular localization of TJ protein at plasma membrane. In mouse Sertoli cells, dexamethasone potentiates development of TER, suggesting regulatory role of glucocorticoid for inter-Sertoli

TJ development.

Follicle stimulating hormone (FSH): FSH has been known to stimulate TER development in cultured Sertoli cells but inhibits claudin-11 expression. Because inter-Sertoli TJ is a multimolecular complex, and paracellular barrier function of TJ is under influence of subcellular localization as well as kinds of TJ proteins, TJ proteins other than claudin-11 may mediate FSH-induced increase in TER in Sertoli cells. Effect of FSH on the expression of the other members of inter-Sertoli TJ should be unraveled for understanding the regulation of BTB by this important gonadotropic hormone.

Functional regulation of Inter-Sertoli TJs by interaction with extracellular matrix: The intriguing interactions of cytokines, such as TGF-βs and proteases/protease inhibitors, such as α2-macroglobulin, metalloproteases (MMPs), in the basement membrane can determine the integrity and homeostasis of ECM, which in turn regulates the BTB dynamics. Extracellular matix is important for the development, maintenance and strength of the inter-Sertoli TJs permeability barrier *in vitro* (Siu *et al.*, 2003).

Functional regulation of Inter-Sertoli TJs by cellular interaction: When the Sertoli cells was placed in the Leydig cells coculture, the TER of Sertoli cells was markedly accelerated and high level of TER was maintained. In co-cultured Sertoli cells, claudin-11 mRNA markedly increased but claudin-1 did not. This suggests that potentiation of inter-Sertoli TJs permeability barrier waslargely attributed to up-regulation of claudin-11 by soluble factors derived from Leydig cells. Therefore, it cannot be excluded that growth factors and cytokines mediating the paracrine interactions between Leydig cell and Sertoli cell might be important for development of inter-Sertoli TJs.

Functional assay for paracellular permeability of inter-Sertoli TJs and thus BTB

Sertoli cell culture: Primary culture of Sertoli cells on ECM could support cell proliferation, differentiation and development of TJ in rodent Sertoli cells. Recent development of Matrigel-coated cell culture plate insert

makes the Sertoli cell culture in polarized condition *in vitro*. It is possible to mimic paracrine interactions between cells in the testis by coculture of Sertoli cells on the insert with other cells in the bottom well.

Monitoring the paracellular barrier property of Sertoli cells: Simple measure of electrical resistance across the culture of cell monolayer reveals the biophysical information on the sealing status of intercellular space by TJ. Paracellular permeability between Sertoli cells also can be monitored by measure of TER. Two electrodes system built in the cell culture plateinsert system is used in the study of TER in Sertoli cells monolayer. Diffusion of fluoroscence-labeled tracer molecules acrossthe epithelial monolayer also has been used to monitor paracellular permeability between Sertoli cells.

In vivo model: Conventionally, intravenous injection of small particles at varying size and tracking the distribution of the particles in the tissue by EM have been used to reveal the paracellular permeability of BTB in animals.

Perspectives

Unraveling the molecular nature and functional regulation of inter-Sertoli TJ is important for understanding the spermatogenesis and development of male contraceptive. On the other hand, possible change in inter-Sertoli TJ by natural and synthetic chemicals such as endocrine disruptors is potentially important for understanding the male reproductive toxicity of certain chemicals. On the other hand, future development of automatic TER monitoring system combined with massive primary culture of Sertoli cells or suitable cell line will decrease labor for studying inter-Sertoli TJ and development for male contraceptive targeted to BTB.

Conclusions

This review summarizes some recent advances in the inter-Sertoli TJ study, in particular the molecular architecture and regulation of functional dynamics by cytokines, steroids, extracellular matrix and cell-cell interaction in testis together with tools for TJ study. To date, however, the genes build up the inter-Sertoli TJ and the plasticity of inter-Sertoli TJ by set of TJ genes at the specific stage of spermatogenesis are not fully uncovered. Further studies on the additional members of TJs and the

factors regulating the assembly of TJ or alter barrier properties of inter–Sertoli TJ may be helpful for manipulation of spermatogenesis based on comprehensive understanding of BTB and spermatogenesis.

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