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THE BLOOD-FOLLICLE BARRIER (BFB) IN DISEASE AND IN OVARIAN FUNCTION

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Abstract

The blood-follicle barrier (BFB) is one of the blood-tissue barriers in mammalian body found in developing follicles in the ovary. The BFB, besides the tight junction (TJ)-permeability barrier of the endothelial cells in the microvessels that surround the developing follicle, is constituted and contributed significantly by the basement membrane of the developing follicle which alters its composition rapidly during follicle development. While the concept of the BFB and its ultrastructure were described more than six decades ago, fewer than 20 reports are found in the literature that were dedicated to investigate the biology, regulation, and function of the BFB either in health or in disease. Furthermore, detailed analysis of the adhesion protein complexes and the regulation of the junction dynamics at the BFB are still missing in the literature. The goal of this short chapter is to provide an update on this important blood-tissue barrier, it is obvious that future investigation is much needed in the field to understand this ultrastructure better in order to treat and better ovarian disorders including ovarian cancer.

INTRODUCTION: THE CONCEPT OF THE BLOOD-FOLLICLE BARRIER (BFB)

Follicular fluid fills the follicular antrum and surrounds the ovum in an ovarian follicle, which in turn provides a unique microenvironment for follicular development and oocyte maturation.¹ Based on the morphological studies conducted in the 1950s and 1960s, it is known that ovarian follicles are surrounded by a network of capillaries in the theca interna, the follicular compartment per se is nonvascular, and each follicle is separated from the surrounding microvessels by a unique basement membrane,^{2–4} illustrating the likely presence of a blood-tissue barrier at the site, which was designated the “blood-liquor barrier” in 1958.⁵ However, the term BFB was first used in the literature in 1973 when the protein composition and concentration between the follicular fluid and blood serum or plasma were found to be different,⁶ consistent with a subsequent report using follicular fluids and serum samples from women undergoing IVF.⁷ This work was further expanded using a proteomic approach, and it was found that human follicular fluid contained several acute inflammatory phase proteins including transferrin, afamin, ceruloplasmin, hemopexin, haptoglobin, and plasma amyloid protein in levels different from the plasma, which also

supported the notion that ovulation is similar to an inflammatory response.⁸ The same study also found some antioxidant enzymes, including superoxide dismutase, glutathione transferase, catalase, paraoxonase, and heat shock protein 27 which can help to protect human follicles from toxic injury mediated by oxidative stress.⁸ It is believed that proteins in the follicular fluid are similar to the blood plasma, providing important growth factors for follicular development and oocyte maturation.⁸ In fact, with respect to low-molecular-weight proteins, the components of follicular fluid is similar to the blood plasma.^{6,9} However, the protein content of human follicular fluid is different from that of the blood plasma due to the selective BFB which serves as a “molecular sieve”.^{6,10} For instance, in a study using ferritin (MW 500 kDa) and colloidal gold (MW 1000 kDa) as tracers to assess the BFB permeability, it was found that BFB served as a molecular sieve which was permeable to proteins < 500 kDa.¹¹ Subsequently, the BFB was found to be both charge- and size selective in mouse ovaries.¹²

COMPOSITION OF THE BFB

In order to transport from the theca into the follicular antrum, proteins/peptides, sugars, electrolytes, and ions in fluid must pass through the BFB which is composed of the vascular endothelium, sub-endothelial basement membrane, the thecal interstitium, the follicular basement membrane, and the membrana granulosa (see Fig. 1).¹³

The Permselectivity of the BFB

A recent study using the ‘in vivo cryotechnique’ (IVCT) to examine mouse ovaries morphologically and immunohistochemically,¹³ it was shown that the immunostaining of albumin (~69 kDa, referred to as a low molecular size protein) was localized in the blood vessels, the interstitium, and developing follicles, suggesting that proteins with low molecular size can pass through the BFB towards developing follicles. While the immunostaining of mid-sized molecules, such as IgG1 (~150 kDa), IaI (inter- α -trypsin inhibitor, 220 kDa), and fibrinogen (340 kDa), were significantly reduced inside ovarian follicles, suggesting that although proteins with middle molecular size can pass through the blood vessels towards the interstitium, the follicular basement membranes inhibit the permselectivity of these molecules. For high molecular weight proteins, such as IgM (~900 kDa), its immunostaining was mostly restricted to the blood vessels, suggesting that proteins with high molecular size are blocked by the endothelial TJ-barrier of blood vessels. These findings suggest that the BFB is present from early stages of folliculogenesis and it has selective roles during follicular development until ovulation.¹³

Endothelial Cells of Blood Vessels

The endothelial cells of blood vessels, mostly capillaries, contribute to the BFB which act as a permeability filter regulating the passage of serum proteins from blood vessels to the surrounding tissue, and such passage is tightly controlled by the dynamic nature of the junctional complexes, such as tight and adherens junctions and desmosomes, between endothelial cells.^{14,15} In the ovary, such as mouse ovaries, the intercellular junctional complexes between endothelial cells are not well characterized, but the blockade of mid-sized proteins by the endothelial TJ-barrier has been shown to be regulated by nitric oxide.¹⁶ It is also noteworthy that the BFB was found to be compromised as the result of a loss of

ovarian superoxide dismutase activity in diabetic mice that led to ovulation defects, illustrating the critical role of reactive oxygen species to the BFB function.

Subendothelial Basement Membranes

Dependent on the pore size and charge selectivity of the components including Type IV collagen, laminin, and heparan sulfate proteoglycans, the basement membrane of blood vessels such as glomerular basement membrane can serve as a molecular sieve.¹⁷

Follicular Basement Membranes

Since the permselectivity of the subendothelial basement membranes and the follicular basement membranes is different, it is suggested that this may be due to the presence of unique extracellular matrix components. During folliculogenesis, the follicular basement membrane is a dynamic ultrastructure with its components rapidly change over time.¹⁸ In primordial and pre-antral stages, collagen IV alpha 1–6 is present and later on in antral and atretic stages, but only alpha 1 and alpha 2 chains are present in the follicular basement membrane in cows¹⁸ and mice.¹⁹ On the contrary, perlecan, nidogen 1 and nidogen 2 are the predominant follicular basement membrane components in the pre-antral stage and thereafter in cows.^{20,21} Laminin alpha 1, beta 2 and gamma 1 are also detected in the follicular basement membrane during follicle development in cows.¹⁸ It is possible that such dynamic changes in the follicular basement membrane components contribute changes to the basement membrane permeability during folliculogenesis.

The BFB in Polycystic Ovary and Ovarian Cancer

Polycystic ovary syndrome (PCOS) is one of the most common female hormonal disorders characterized by anovulation, leading to irregular menstruation, infertility and polycystic ovaries.^{22,23} In PCOS, follicle development is put on hold at an early follicular stage.²⁴ Since BFB regulates the composition of the follicular fluid by determining its component proteins, which in turn regulates follicle development, PCOS can be affected, at least in part, by the permselectivity of the BFB. Previous studies have shown that insulin-like growth factor binding proteins,²⁵ soluble Fas ligand²⁶ and inhibins A and B²⁷ are dysregulated in the follicular fluids in PCOS patients.

Using the IVCT in a mifepristone-induced PCO model, the morphology and the permselectivity of the BFB was examined in mouse ovaries,²⁸ in which the blood vessels were found to be enlarged along with an increase in blood flow, and follicular cysts were formed with thinner membrana granulosa in the blood vessels, the interstitium, and developing follicles.²⁸ The immunostaining of low and high molecular weight proteins, albumin and IgM, respectively, in the ovaries of PCO model mice were similar to that in normal mice. Albumin was detected in the blood vessels, the interstitium, and developing follicles and IgM was mostly retained inside the blood vessels.²⁸ On the contrary, the immunostaining of mid-sized molecules such as IgG, ITI, and fibrinogen had different pattern in the PCO model. The follicular basement membranes blocked the passage of both IgG and ITI from the interstitium to the follicles in PCO model. Moreover, fibrinogen was mostly restricted within the blood vessels and surrounded by the endothelial cells in the PCO mice.²⁸ These findings suggest that the permselectivity of BFB mediated by the

endothelial cells of the microvessels and the follicular basement membrane may play important role in the pathogenesis of PCOS.²⁸

Ovarian cancer is the most lethal of all gynecological malignancies.^{29,30} Approximately 90% of the malignant ovarian tumors are originated from changes in the surface epithelium or surface epithelial inclusion cysts of the ovary.^{31,32} It is possible that dysregulation of the BFB contributes to: (i) the abnormal changes of the surface epithelium, and/or (ii) the formation of surface epithelial inclusion cysts, of the ovary, which, in turn, leads to cancer progression. This possibility regarding the role of BFB in ovarian cancer must be carefully evaluated in future studies.

THE PERMEABILITY BARRIER FUNCTION OF THE BFB: SELECTIVE TRANSPORT PROCESSES VERSUS MERE FILTRATION

Similar to other blood-tissue barriers, such as the blood-brain barrier and the blood-testis barrier,^{33–36} the BFB is now known to be more than a “molecular sieve” in the ovary by restricting the transcellular transport of solutes and macromolecules across the various membranes ultrastructures to reach the developing follicles entirely based on their molecular sizes. For instance, IαI (inter-α-trypsin inhibitor, 240 kDa) and pre-α-trypsin inhibitor (125 kDa) were found to be absent from the follicular fluid until an ovulatory stimulus (e.g., during LH surge) was given, and both of these protein were found in the antrum of mature follicles, usually within minutes, and integrally associated with the newly synthesized hyaluronic acid-rich cumulus extracellular matrix.^{37,38} Subsequent studies have shown that these changes in permeability function at the BFB are also mediated by nitric oxide (NO),¹⁶ consistent with earlier findings that NO regulates vascular function and permeability^{39–41} as well as the Sertoli cell TJ-barrier permeability function.⁴²

CONCLUSION AND FUTURE PERSPECTIVES

As briefly summarized above, the BFB is an important ultrastructure in the ovary by limiting the access of foreign compounds and/or harmful substances (e.g., toxicants, drugs) to the developing follicles. It is intimately related to follicle development and it also regulates the composition of the follicular fluid via subtle changes in its basement membrane composition during folliculogenesis, so that different components (e.g., proteins, peptides, electrolytes, ions, sugars, and others) can be precisely and rapidly recruited to the follicular fluid in response to the needs of developing follicles. A recent study has shown that some therapeutic drugs, such as doxorubicin, an anticancer drug, can permeate the BFB to induce ovarian failure by causing apoptosis of germinal vesicle oocytes,⁴³ illustrating much work is needed to understand this blood-tissue barrier so that the reproductive health of women under chemotherapy can be maintained. While the concept of BFB was depicted more than six decades ago and the term BFB was used almost 40 years ago, yet, less than 20 reports are found in the literature dedicated to study the biology and regulation of the BFB. It is likely that proteins found in the follicular fluid behind the BFB can be putative candidates for diagnostic markers for follicle and/or oocyte maturation and to assess oocyte quality. It is equally possible that follicular fluid proteins can serve as diagnostic, therapeutic and/or prognostic markers for various ovarian diseases including PCOS and ovarian cancers. There

are pressing questions remain to be addressed. For instance, what initiates or regulates the formation of follicular fluid? Does this involve LH and/or FSH? What are the changes in cell-cell junctions that facilitate the formation of follicular fluid during folliculogenesis?

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Figure 1.
Schematic drawing illustrating the different components that constitute the blood-follicle barrier.