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Cell-Cell Interactions that Control Spermatogenesis and Oocyte Maturation

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INTRODUCTION

The evolution of multicellular organisms was prompted by the establishment of cell-cell communication. For this reason in a multicellular organism no cell type is autonomous and a complex network of cell-cell interactions are needed during development and in the regulation of normal adult tissue function. This concept of cell-cell interactions and homeostasis was originally proposed by Claude Bernard in 1878 (2,3). Today the mechanistic aspects of these cell-cell interactions are starting to be elucidated. A unique cell type in most higher organisms is the germ cell undergoing the process of gametogenesis. The germ cell is distinct from other cells (i.e. somatic cells), but is dependent on somatic cell support for survival and development. In vertebrates multiple somatic cell types are required to support germ cells and the process of gametogenesis. Because viable germ cells are essential for the propagation of the species, a number of critical cell-cell interactions have evolved to maintain the process of gametogenesis. This paper will provide a brief survey of several important cell-cell interactions involved in the control of spermatogenesis and oogenesis.

The testis is composed of a number of somatic cell types to support the process of spermatogenesis. The Sertoli cell is an epithelial-like somatic cell that forms the seminiferous tubule and provides the physical support and microenvironment for the developing spermatogenic cells. The peritubular myoid cells are a mesenchymal

cell that surrounds the tubule and is separated from the basal surface of the Sertoli cells by a complex basement membrane. Within the interstitium are the Leydig cells that are responsible for the production of androgens. The interstitium also has macrophages, lymphatic epithelium and vascular endothelium. These cell populations organize and differentiate during embryonic and prepubertal testis development. At the initial stage of embryonic testis determination the Sertoli cells form cords that migrating germ cells localize into and eventually develop into seminiferous tubules postpubertally. The peritubular cells localize around the cords embryonically and the Leydig cells develop from the embryonic gonadal mesenchyme. The spermatogonia proliferate prepubertally and the process of spermatogenesis is initiated at the onset of puberty when the lumen of the tubule and blood-testis barrier form. The primary somatic cells that support the developing spermatogenic cells are the Sertoli, peritubular, and Leydig cells.

The ovary is also composed of a number of somatic cell types to support the process of oogenesis and oocyte maturation. The granulosa cell is the epithelial cell that provides the physical support for the developing oocyte within the ovarian follicle. The theca cells surround the outer layer of mural granulosa cells and are separated by an extracellular matrix. Theca cells are mesenchymal cells that form the outer cellular layers of the developing follicle. Between follicles is a stromal-interstitial cell population that also has vascular endothelium present. During embryonic development clusters of germ cells proliferate and eventually precursor granulosa cells form a single layer around the individual germ cells. At the onset of puberty and throughout the female reproductive period primordial follicles will enter the process of folliculogenesis and start to develop. Initially the theca cells are recruited from the stromal-interstitial stem cell population. As the follicle develops both the granulosa and theca cells proliferate. Follicles will eventually form an antrum and develop into an ovulatory follicle to eventually release a cumulus-oocyte complex. However, the vast majority of follicles undergo atresia and degenerate at any stage of folliculogenesis. The primary somatic cells that support the developing oocyte are the theca and granulosa cells.

As specific cell-cell interactions are discussed they will be categorized as environmental, nutritional or regulatory interactions as previously described (30). Environmental interactions are

mediated by extracellular factors such as extracellular matrix or cell adhesion molecules that influence the physical support and structural cytodifferentiation of the cell. Nutritional interactions are mediated by a nutritional substance or enzyme metabolite between cells to support cell metabolism and viability. Regulatory interactions are mediated by paracrine factors that through a receptor mediated signal transduction event modulate cellular functions, growth and differentiation on a molecular level. These categories are useful to functionally distinguish various types of cell-cell interactions.

SOMATIC-GERM CELL INTERACTIONS

Testis Interactions

In the testis the somatic-germ cell interactions occur between the Sertoli cells and spermatogenic cells. The spermatogenic cells develop from a stem cell population of spermatogonia on the basal surface of the tubule through meiosis and are released into the lumen of the tubule as a spermatozoa. This physical support of the spermatogenic process is provided by the Sertoli cell. This environmental interaction between the Sertoli and spermatogenic cells is one of the most complex cytoarchitectural arrangements of any cell types known. An appreciation of this environmental interaction was shown through a 3-dimensional reconstruction of the Sertoli cell (33) demonstrating that a single Sertoli cell is in contact with as many as 50 different germ cells. The fact that the developing germ cells exist in a synsistium that is maintained until elongate spermitides are released add to the complexity of this environmental interaction. This Sertoli-spermatogenic cell interaction is essential to maintain the proper cytoarchitecture and physical support required for the process of spermatogenesis. A number of different junctional interactions and cytoskeletal parameters are involved in this environmental interaction (23).

At the onset of puberty tight junctional complexes form at the basal regions of the tubule between Sertoli cells. These tight junctions create a blood-testis barrier such that the developing spermatogenic cells are sequestered in a serum free microenvironment within the tubule. The Sertoli cell provides a microenvironment within the tubule to support the nutritional requirements of the developing

spermatogenic cells. Because serum components are not available to the spermatogenic cells, Sertoli cells produce a wide variety of nutrient binding proteins including metal binding proteins, vitamin binding proteins and lipid binding proteins (12,30). The nutrients are delivered to the basal surface of the Sertoli cell by serum components. These substances are then transferred to Sertoli binding proteins within the Sertoli cell. These Sertoli binding proteins are then secreted and deliver the nutrients to the spermatogenic cells. As an example, serum transferrin transports iron to the basal surface of the Sertoli cell that is then endocytosed and delivers iron to testicular transferrin that is secreted by the Sertoli cell within the blood-testis barrier to deliver iron via transferrin receptors on the spermatogenic cells to be utilized by the germ cells for cytochrome enzymes and respiration (12,24,30). The transport of these essential components to the spermatogenic cells is a critical nutritional interaction between these cells required to maintain the process of spermatogenesis.

Regulatory interactions between Sertoli cells and spermatogenic cells can be mediated by paracrine factors (30). Although not many specific factors have been identified to date (30), several do appear to be critical. One of the best examples is the production of kit-ligand (stem cell factor) by Sertoli cells (15) and actions at the ckit receptor on spermatogonia (8,14). The kit-ligand appears to be essential for the proliferation of early spermatogonia and this is controlled by Sertoli production of kit-ligand. Spermatogenic cells have been shown to produce nerve growth factor (NGF) that is postulated to act at NGF receptors on Sertoli cells to regulate Sertoli function (20). Interleukin mediated interactions between Sertoli cells and spermatogenic cells have also been postulated (11,32). Clearly regulatory interactions between Sertoli cells and spermatogenic cells will be important to maintain the process of gametogenesis. The further identification of specific paracrine factors that mediate this cell-cell interaction will be an active area of research to come.

Ovary Interactions

In the ovary the somatic-germ cell interactions occur between the granulosa cells and oocyte. The oocyte is arrested in meiosis and expands in size during follicle development. The oocyte is in physical contact with a layer of granulosa cells from the primordial follicle stage, throughout follicle development and even after ovulation. This environmental interaction between the granulosa

cells and the oocyte is essential for the physical support of the oocyte in the developing follicle, as well as help maintain the integrity of the oocyte after ovulation. Complex junctional contacts are formed between the oocyte and granulosa (9). These junctional contacts provide a direct cell communication and are involved in the environmental interaction between these cells. As the zona pelucida forms around the oocyte these junctional contacts are maintained and required for oocyte maturation. As the layers of cumulus granulosa cells develop around the oocyte they are supported in the form of a stock by the antral granulosa cells. Therefore, environmental interactions between granulosa and the oocyte are required for the maintenance of oocyte maturation and structural integrity of the oocyte.

The junctional complexes that form between the granulosa cells and the oocyte can allow the passage of nutrients <700-1000 MW. Therefore, the granulosa can provide nutrients to the oocyte and cooperate metabolically. In contrast to the testis, the ovarian follicle is not a serum-free environment. Follicular fluid is an ultrafiltrate of serum and granulosa products. These components can directly interact with the oocyte. Therefore, the essential nutritional needs for a somatic-germinal cell interaction in the testis are not required in the ovary. It is likely that important nutritional granulosa-oocyte interactions are required, however, specific components involved remain to be identified.

Several important regulatory interactions between granulosa cells and the oocyte have been identified. The first is a unique regulatory interaction involving cAMP and the junctional complexes between the cells. The junctions allow the passage of cAMP and maintain a high level of cAMP in the oocyte. This level of cAMP appears to be required to maintain meiotic arrest (10). Gonadotropin actions on the granulosa indirectly maintain the high level of cAMP in the oocyte through the junctional complexes. As ovulation occurs and the granulosa cells leutinize the junctional complexes break down and the cAMP levels in the oocyte drop. This causes germinal vesicle breakdown and the resumption of meiosis. A more classical regulatory interaction via a paracrine factor is through the production of kit-ligand by granulosa cells and actions of the kit-ligand at the ckit receptors on the oocyte (13,18). Granulosa cells appear the primary source of the ligand and the oocyte has the receptor and can respond to kit-ligand to grow (18). Other factors such as IGF-1, TGFα and TGFβ

have also been postulated to mediate granulosa-oocyte interaction (31). The identification of specific factors that mediate this cellular interactions is currently an active area of research.

Hormone Action

It appears that the ability of hormones to influence gametogenesis is indirectly mediated through the somatic cells. For example, the actions of FSH on Sertoli and granulosa cells indirectly regulates germ cell development. Germ cells do not appear to be the site of hormone actions and the ability of somatic cells to respond to hormones is one reason essential somatic-germ cell interactions evolved. Many of the specific interactions yet to be identified will likely be directly involved in mediating hormone actions. Since critical environmental, nutritional and regulatory interactions exist between the Sertoli-spermatogenic cells and the granulosa-oocyte, anything that regulates Sertoli and granulosa cell function will influence germ cell development. Therefore, the other cell types in the gonad through interactions with Sertoli and granulosa cells can also have a critical role in gametogenesis.

SOMATIC-SOMATIC CELL INTERACTIONS

Testis Interactions

Although a number of different cell types are present in the testis, the Leydig cell and peritubular myoid cell appear to directly influence Sertoli function and will be discussed. The Leydig cells in the interstitium is the major site of androgen production in the male. Although Leydig cells can produce other components that may influence seminiferous tubule function (30), androgens are essential for testis function and provide a major regulatory interaction with peritubular cells and Sertoli cells. The Leydig cell does not have direct contact with peritubular myoid cells or Sertoli cells so does not have an environmental interaction with these cells. All these cells have direct access to serum components so nutritional interactions also are not important between these cells. Regulatory interactions do exist through the production of androgen (30). Androgen receptors are present in both peritubular cells and Sertoli cells (1). Although Sertoli cells contain androgen receptors, highly purified

populations of Sertoli cells do not respond directly to androgens with a number of major functional parameters examined (30). Sertoli cells do have the androgen receptor so have the capacity to respond directly to androgens. Further research is needed to elucidate what functional parameters are directly regulated by androgens. Peritubular myoid cells also contain the androgen receptor. Androgens appear to be required for peritubular cell differentiation prepubertally (4). As discussed below androgen responsive paracrine factors can regulate Sertoli cell functions. Sertoli cells also have the capacity to produce factors that regulate Leydig cell function, however, these factors remain to be elucidated (30). Therefore, Leydig cells have important regulatory interactions with peritubular cells and Sertoli cells.

The peritubular myoid cell is a mesenchymal cell surrounding the seminiferous tubule. Between the peritubular cell and the Sertoli cell is a basement membrane produced cooperatively by the cells (25) that has effects on Sertoli cell cytoarchitecture (7). This interaction between peritubular and Sertoli cells via this basement membrane is a critical environmental interaction between the cells. Both these cells have access to serum so nutritional interactions do not appear to be critical (30). During development the association of peritubular cells and Sertoli cells forms early when cords are formed. Peritubular cells may in part be due to migration of mesenephric cells into the embryonic gonad (5). Therefore, peritubular-Sertoli interactions may be needed throughout testis development. Regulatory interactions between peritubular cells and Sertoli cells have also been demonstrated (30). Under androgen control peritubular cells produce a factor termed PModS (26) that has dramatic effects on Sertoli cell differentiation (29) through a critical tyrosine phosphorylation event (17). Recently PModS was found to promote early event genes that subsequently regulate downstream Sertoli cell differentiated functions (e.g. transferrin expression) (16). One of the key transcription factors has been identified as a basic helix-loop-helix factor that acts at a unique response element on the transferrin promoter. PModS appears to act as an important regulator of Sertoli differentiation and provides a mode of androgen action in the testis. It is likely that other paracrine factors will also mediate peritubular-Sertoli interactions that remain to be identified (30). Therefore, both environmental and regulatory interactions are involved in peritubular-Sertoli interactions.

Ovary Interactions

A number of different cell types also are present in the ovary, however, the theca cells appear to directly influence granulosa cell function and will be discussed. Theca cells and granulosa cells are separated by an extracellular matrix. Therefore, environmental interactions exist between the cells. This helps organize the outer layers of mural granulosa cells and inner layers of theca interna cells during follicle development and maintain structural integrity for the follicle. At the onset of development of a primordial follicle theca cells differentiate from the stromal-interstitial cell population between follicles. A close association of theca and granulosa then continues throughout follicle development. Both theca and granulosa have access to serum components and the cells do not have direct junctional contacts. Therefore, general nutritional interactions are not likely important between the cells. However, a classic nutritional interaction exists involving steroid synthesis. Under the control of LH theca cells during early follicle development produce androgens that are utilized as a substrate for aromatase in granulosa cells for estrogen production (6). This is the two cell hypothesis in that granulosa cannot produce androgen and require theca androgen production. This is a critical nutritional interaction between theca and granulosa cells.

Regulatory interactions between theca and granulosa cells have also been demonstrated. Although the theca supply of androgen to granulosa is a nutritional interaction, the estrogen produced by the granulosa has been shown to feedback on the theca cells to regulate androgen production (21). This adds to the complexity of the two cell theory and is an example of a regulatory interaction between the cells. Theca cells have also been shown to produce a number of paracrine factors that influence granulosa cells. Theca cells produce transforming growth factor-alpha as a growth stimulator for granulosa (28). In contrast, theca cells also produce transforming growth factor-beta as an inhibitor of granulosa growth (27). The inverse actions of TGFα and TGFβ provide an efficient growth control mechanism for the developing follicle (22). Recently, theca cells have also been shown to produce two mesenchymal growth factors keratinocyte growth factor and hepatocyte growth factor that both can stimulate granulosa growth (19). Therefore, theca cell produce a number of paracrine factors that can influence follicle growth. Interestingly this may be a mesenchymally controlled growth process.

It is likely a large number of other factors will participate in theca-granulosa cell interactions yet to be identified. Clearly, this is a critical regulatory interaction in the ovary.

SUMMARY

Cell-cell interactions in the gonads that are essential for gametogenesis are numerous. Environmental interactions are critical for somatic-germ cell interactions. These interactions provide the physical support and cytoarchitecture required for germ cell development. Nutritional interactions are extensive and essential between Sertoli cells and spermatogenic cells. This has evolved in large part from the presence of the blood-testis barrier and is a major function of the Sertoli cell. The cooperation of theca cells and granulosa cells in estrogen production is also a critical nutritional interaction. Therefore, nutritional interactions are tailored to the specialized physiologies of the tissues. Regulatory interactions are extensive between all the cells. More research is needed to elucidate how somatic cells and germ cells directly interact. This will be initially through the further identification of specific paracrine factors. The Sertoli-spermatogenic or granulosa-oocyte regulatory interactions will likely be critical in maintaining the process of gametogenesis. Another critical regulatory interaction identified is a mesenchymal-epithelial cell interaction in both the testis and ovary. The peritubular-Sertoli and theca-granulosa interactions both were found to be extensive and important for the maintenance of gonadal function. This supports a large body of literature that mesenchymal cells have an important role in promoting and maintaining the differentiation of adjacent epithelial cells. Therefore, these functions appear to be important throughout development and in the adult. The testis is a steady state optimally differentiated tissue so the peritubular-Sertoli interactions identified appear focused on maintaining optimal differentiation. In contrast, the ovarian follicle is very dynamic and growing so the theca-granulosa interactions appear focused on cell growth. Further analysis of these mesenchymal-epithelial interactions will likely provide further insight into the factors regulating gametogenesis.

In conclusion the hormonal regulation of gonadal function several observations have been made. The first is that hormones regulate

gametogenesis indirectly through the somatic cells. This has likely been a driving force in the evolution of specific somatic-somatic cell interactions in the gonads. This supports the proposal that no cell type is autonomous and even the endocrine regulation depends on a cascade of local cell-cell interactions. The future research regarding the endocrinology of the testis and ovary will involve an elucidation of cell-cell interactions that mediate the actions of specific hormones.

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