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Growth Factor Regulation of Testicular Function

Brian P. Mullaney and Michael K. Skinner

Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee

The evolution of multicellular organisms required communication between different cells to coordinate tissue function. These cell-cell interactions have become vital for the regulation of cellular function, growth, and differentiation.

CELL-CELL INTERACTIONS IN THE TESTIS

The testis provides a useful model system to study cell-cell interactions due to the presence of a variety of cell types and the local production of regulatory factors. Testicular cell-cell interactions between Leydig, peritubular, Sertoli, and germ cells are important in the regulation of testis function and the process of spermatogenesis (1). Interactions between these different cell types may be categorized into environmental, nutritional, and regulatory interactions. Environmental interactions are mediated by such components as extracellular matrix and cell adhesion molecules. For example, Sertoli cells provide the proper microenvironment and cytoarchitectural support for the developing germinal cells. Peritubular-myoid cells contribute to the exterior wall of the seminiferous tubule and are separated from Sertoli cells by an extracellular matrix. This extracellular matrix and tight junctions between Sertoli cells form the blood-testis barrier. Therefore, Sertoli cells have nutritional interactions with germinal cells through the production of transport proteins necessary for the delivery of essential metabolites across the blood-testis barrier. Regulatory type cell-cell interactions are also present in the testis and are mediated by paracrine factors via receptor-mediated signal transduction events. Growth factor regulation of cell growth and differentiation are considered regulatory type interactions. This chapter briefly reviews growth factor-mediated cell-cell interactions in the testis.

TESTICULAR GROWTH AND DEVELOPMENT

Growth regulation is necessary for the development of the testis and maintenance of spermatogenesis. In the prepubertal testis Sertoli cells divide and grow to form the seminiferous tubule. The growth of these cells arrests at early puberty, and these cells become terminally differentiated. Peritubular-myoid cells appear in late fetal development and further proliferate. Similarly, Leydig cells also appear in late fetal development, but then degenerate and may further proliferate with the coincident initiation of spermatogenesis. Germinal cell development begins shortly after birth when gonocytes mitotically divide, forming spermatogonia. Developing spermatogonia traverse the blood-testis barrier and mature. Postpubertal growth control of somatic and germinal cell types is also necessary. At the onset of puberty, germinal cell meiosis begins, and the developing spermatozoa become the most abundant cell type in the seminiferous tubule. Proliferation of somatic and germ cells may require growth factors; however, other signals must be present to terminate growth and initiate differentiation. For example, growth inhibitory factors must be responsible for halting Sertoli cell growth and stimulating differentiation. Therefore, both positive and negative growth regulation appear to be necessary for the development of testis function.

GROWTH FACTORS IN THE TESTIS

A complex variety of growth factors appears to be required for growth regulation in the testis (2). Seminiferous growth factor (SGF) was the first mitogenic factor identified in the tubule (3–4). Although this protein has not been fully characterized, initial biochemical studies suggest it is not a previously identified growth factor, but this remains to be thoroughly investigated. The presence of the blood-testis barrier may require local production of essential factors for germinal cell division due to the exclusion of agents normally found in serum. One such factor, insulin-like growth factor I (IGF-I), has been identified in the testis (5). Immunological evidence suggests that Sertoli cells produce IGF-I, while receptors are present on Leydig, Sertoli, and germinal cells (7). IGF-I can stimulate cell function, as indicated by increased Leydig cell steroidogenesis and Sertoli cell transferrin production (8–10). Another general growth factor, basic fibroblast growth factor (bFGF), may locally regulate gonadal function (11–12). Studies indicate bFGF is mitogenic for immature porcine Sertoli cells (13–14). A neurotrophic factor, beta-nerve growth factor (β-NGF), is also expressed in the testis (15). NGF mRNA has been detected in developing germ cells, while NGF receptor message may be present on Sertoli cells (16). Testosterone down regulates NGF receptor mRNA levels in vivo (16). These observations suggest a potential germ cell-Sertoli cell interaction. Surprisingly, immunological growth peptides, namely, an interleukin-like factor (IL-I), have also been detected in the testis (17). IL-I may regulate growth or immune suppression in the seminiferous tubule. More recently, transforming growth factors alpha and beta (TGFα and TGFβ), growth regulators with antagonistic actons, have been shown to be produced locally in the testis (Fig. 1).

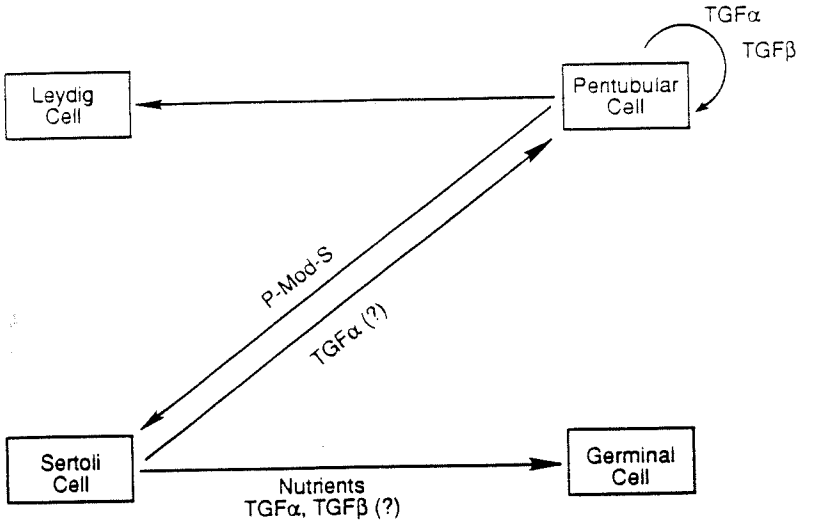

Fig. 1. Potential transforming growth factor-mediated cell-cell interactions in the testis.

Epidermal growth factor (EGF) has been implicated in the maintenance of spermatogenesis (18). Sialoadenectomized mice exhibited a 50% reduction of mature sperm, while EGF replacement returned spermatogenesis to normal levels. However, low circulating concentrations of EGF appear to imply the local production of an EGF-like factor. Local production of an EGF-like substance in the testis was initially supported by a report that Sertoli cells secrete a factor that blocks EGF from binding to its receptor (19). A candidate for mediating these effects is TGFα. TGFα is a peptide that has high homology with EGF, acts at the EGF receptor, and can mimic the actions of EGF (20). Therefore, the potential local production of TGFα as the EGF-like substance in the testis was examined. TGFα expression and protein production were found in midpubertal Sertoli and peritubular cells, but not in a crude mixed population of germ cells (21). Analysis of the potential sites of action of TGFα in the testis was investigated by localization of the EGF receptor. Scatchard analysis revealed high-affinity (100-pM) EGF receptor-binding sites on peritubular cells, but no EGF receptors were detected on midpubertal Sertoli cells (21). In contrast, another report presents immunological evidence that Sertoli cells may contain EGF receptors (22). Experimental limitations to be considered include the sensitivity of binding analysis, antibody crossreactivity, or possible expression of a nonfunctional truncated form of the receptor. The possibility that Sertoli cells contain EGF receptor is currently being examined with molecular probes for the receptor. The literature also suggests that EGF can alter Sertoli cell functions, such

as lactate, inhibin, and estrogen production (23–24). Due to potential interactions between peritubular and Sertoli cells, some observed effects may be mediated through the peritubular cell contaminant of Sertoli cell preparations. For example, EGF can stimulate transferrin production in Sertoli-peritubular cocultures, but not in pure Sertoli cell cultures (21). Therefore, analysis of the actions of EGF/TGFα on Sertoli cell function requires further investigation.

To define further the growth role of TGFα, developmental studies were initiated on prepubertal, midpubertal, and mature isolated cells of the rat testis. Initial studies revealed that TGFα was expressed by both peritubular and Sertoli cells. TGFα can stimulate peritubular cell growth at all stages of development, as indicated by [^{3}H]thymidine incorporation into DNA and increases in cell number. However, neither adult nor immature Sertoli cells respond to TGFα, suggesting that another mitogen is responsible for immature Sertoli growth. Thus, peritubular cell proliferation may be jointly controlled by both peritubular cells and Sertoli cells. Observations have also demonstrated that Leydig cells are responsive to EGF and contain the EGF receptor. Therefore, EGF-mediated seminiferous tubule-Leydig cell interaction is a potential interaction that remains to be investigated. The role of TGFα in Sertoli cell-germ cell interactions is also unclear at present. If developing spermatogonia contain EGF receptors, however, Sertoli cell production of TGFα would provide an appropriate mechanism for initiating spermatogonial growth.

Growth inhibition also appears to be important in maintaining testis function. Terminal differentiation of Sertoli cells requires inhibition of Sertoli cell growth. In addition, the tightly regulated growth of germinal cells may also involve negative growth regulation. The presence of growth inhibitors may be necessary in the control of testis function. Therefore, the potential action of the growth inhibitor TGFβ in the testis was investigated. In comparison with TGFα, TGFβ is a multifunctional regulatory molecule. TGFβ generally inhibits EGF/TGFα-induced cell proliferation. TGFβ can also promote cellular differentiation, stimulate extracellular matrix production, and induce chemotaxis (25). Initial studies suggested that TGFβ-like proteins are produced by Sertoli cells, and their secretion may be modulated by gonadotropins (26). Northern analysis indicates that peritubular and Sertoli cells express TGFβ genes (27). The growth inhibitory action of TGFβ was examined in developmental studies. TGFβ can inhibit TGFα-stimulated peritubular cell growth in all stages of development, while having no effect on immature Sertoli cell growth. Local production of TGFβ may be a mechanism to limit TGFα-induced proliferation (27). The potential role of TGFβ to control the proliferation of spermatogonia in the adult and perhaps prevent prepubertal spermatogenesis remains to be investigated. The role of TGFβ as a differentiation factor is also being examined. Leydig cell steroidogenesis is decreased by TGFβ (28), while TGFβ has no effect on adult Sertoli cell function, including transferrin production (27). However, TGFβ may be important in peritubular cell differentiation. TGFβ can stimulate the production of several high molecular weight proteins, possibly matrix components, by peritubular cells. In vitro, TGFβ induces migration and colony formation of peritubular and Sertoli-peritubular cell cocultures (27). Therefore, morphogenesis and structural formation of the seminiferous tubule may be

dependent on this factor. TGFβ-stimulated chemotaxis may also be a mechanism to recruit nondifferentiated peritubular cells to the exterior of the tubule during development. These observations imply that TGFβ may play an important role in cell-cell interactions in the testis (Fig. 1).

CELLULAR DIFFERENTIATION VERSUS GROWTH

While control of growth is necessary for development of testis function, control of differentiation is vital for development of specialized cellular function. A developing hypothesis is that the control of growth and differentiation are inversely linked. Thus, growth factors may act to shift the cell from a differentiated state to a less-differentiated growth state. For example, EGF and FGF decrease Leydig cell steroidogenesis, and EGF down-regulates hCG receptors (29–30). Therefore, growth factors appear to shift the cell away from its androgen-producing differentiated state, resulting in cell proliferation. Promotion of cellular differentiation will likely involve other nonmitogenic differentiation factors. As an example, a potential testicular differentiation factor in the seminiferous tubule, PModS, is produced by peritubular cells and modulates Sertoli cell differentiation (31). PModS enhances the majority of Sertoli cell functions, such as transferrin and inhibin production (32). PModS can stimulate Sertoli cell function to a greater extent than other known hormones, including FSH. This stimulation is due in part to a unique signal transduction mechanism involving cGMP (33). The production of PModS is regulated by androgens, suggesting a potential indirect mechanism for androgen action in the testis. This paracrine factor is postulated to be essential for the maintenance and control of normal testis function. Therefore, the postulate is made that growth factors may influence growth and development of the testis, while local production of differentiation factors may be important in maintaining testis function.

The growth and development of the testis involves differential growth of a number of cell types, including mesenchymal, epithelial, and germinal cells. In order to coordinate the temporal growth of these cells, a number of factors may be involved. Presently, a variety of growth factors have been identified in the testis. These factors include TGFα, TGFβ, SGF, IGF-I, FGF, NGF and IL-I. Local production and action of these factors may regulate the complex process of cell growth and differentiation. A controlled balance of growth and differentiation factors will likely be important in the development and maintenance of testicular function.

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DAVID W. SCHOMBERG

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David W. Schomberg, PhD
Duke University Medical Center
Durham, NC 27710
USA

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