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Growth Factors in Endocrinology

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Growth factors as mediators of testicular cell–cell interactions

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INTRODUCTION

Tissue growth and development is an essential step in establishing the specialized function of an endocrine organ. Classical endocrine agents like growth hormone can broadly mediate tissue growth; however, precise growth control may occur locally involving the cell types that compose the tissue. One cell may influence the growth of another cell by the production of peptide factors which can act as autocrine or paracrine regulatory agents to alter cellular growth, differentiation and function. The testis is an example of an endocrine organ where locally produced growth factors may be important. The major growth factors identified in the testis and their potential role in mediating cell–cell interactions will be reviewed.

Spermatogenesis occurs within the seminiferous tubules that are composed of a variety of cell types. Sertoli cells form the tubule, create the blood–testis barrier, and provide the proper structural support and nutritional microenvironment for germinal cell development (Griswold, 1988). The mesenchymal (i.e. stromal) derived peritubular myoid cells surround and contribute to the structural integrity of the tubule and are separated from the epithelial Sertoli cells by a complex extracellular matrix. In the interstitium, Leydig cells produce androgens necessary for testis function. Other cell types present in the interstitial tissue include macrophages, fibroblasts and lymphatic endothelial cells. Each of these cell types can produce growth factors which may be important in local cell–cell interactions. The current review will deal primarily with Leydig, peritubular, Sertoli and germinal cells.

Precise growth regulation is necessary for the development of the testis and maintenance of spermatogenesis (Clermont and Perey, 1957). During fetal development all testicular cell types proliferate. The somatic Leydig, peritubular and Sertoli cells continue to actively grow in the prepubertal testis. Sertoli cells in the prepubertal testis actively divide and form the seminiferous tubule (Nagy, 1972; Orth, 1982). The development and differentiation of the Sertoli cell appears to be dependent on the gonadotrophin follicle-stimulating hormone (FSH) (Ritzen et al, 1989). At early

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Table 1. General properties of growth factors identified in the testis.

Growth factor	Approximate size (kDa)	Precursor	Major source	Cellular action	Examples of physiological action
IGF-I	7.5	Secreted 150aa	Most cells	Growth/differentiation	Skeletal growth
IGF-II	7.5	Secreted 180aa	CNS	Growth	Fetal development
TGF-α/EGF	5	Membrane-bound 160aa	Transformed and normal cells	Growth	Tissue growth
TGF-β	25/dimer	Secreted 400aa	Most cells	Growth inhibition/ differentiation	Tissue repair
FGF	17	—	Many cells	Growth	Angiogenesis, tissue repair
NGF	13	αβγ complex	CNS	Growth	Neuronal development and maintenance
IL-1	17	Secreted 279aa	Immune cells	Growth/differentiation	Immune response, inflammation

CNS, central nervous system; EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF, insulin-like growth factor; IL, interleukin; NGF, nerve growth factor; TGF, transforming growth factor.

puberty Sertoli cells cease to divide and terminally differentiate (Orth, 1982). The Sertoli cell may require stimulatory growth factors for prepubertal growth followed by growth inhibitors to halt cell growth and stimulate pubertal differentiation. Peritubular–myoid cells first appear in late fetal development and the majority of peritubular proliferation may occur during formation of tubules. Peritubular cells, however, appear to continue to slowly proliferate in the adult with a defined turnover rate (Teerds et al, 1989). The Leydig cell is thought to arise from the same embryonic mesenchyme as peritubular cells and this cell exhibits a similar developmental growth pattern (Lording and de Kretser, 1972). Leydig cells appear in late fetal development and continue to grow and differentiate before puberty. Leydig cell growth is slowed in the adult, although these cells may actively regenerate after exposure to cytotoxic agents like EDS (ethylene-1,2-dimethane sulphonate) (Hardy et al, 1989; Teerds et al, 1989). Thus, both Leydig and peritubular cell growth may require continuous growth regulation in the adult. In comparison to the somatic cells of the testis, germinal cells exhibit a delayed growth pattern. Some germinal cell development begins shortly after birth when gonocytes mitotically divide forming spermatogonia. At the onset of puberty, germinal cell mitosis and meiosis begins, initiating 'waves' of spermatogenic cell proliferation. In order to co-ordinate this developmental process, growth inhibition may be necessary to prevent prepubertal germ cell growth, while growth stimulation may be needed to initiate spermatogenesis.

Cell–cell interactions between testicular cell types may be categorized into environmental, nutritional and regulatory types (Skinner, 1987). Growth factor regulation of cell growth and differentiation can be considered a regulatory interaction. Certain criteria must be met for evaluation of growth factors in testicular cell–cell interactions. An important growth factor must be produced locally within the tissue and its site of expression, synthesis, secretion and action must be determined. The physiological significance of a growth factor must eventually be investigated in vivo. The most common approach to studying cell–cell interactions involves culture of freshly isolated cells; however, careful interpretation of in vitro experiments is necessary. Variables associated with cell culture include purity of the cell population utilized and culture additives like matrix components which may contain detectable amounts of growth factors (Taub et al, 1990). Presently, few of the proposed testicular cell–cell interactions involving growth factors have evaluated all these criteria. The complex co-ordination of testicular development and function suggests that a variety of factors may be involved (Table 1) (Bellve and Zheng, 1989). This review will attempt to briefly discuss these growth factors and classify their potential role in mediating interactions between specific testicular cell types.

SERTOLI CELL–GERMINAL CELL INTERACTIONS

Growth factor interactions between Sertoli and germinal cells may co-ordinate the process of spermatogenesis (Griswold et al, 1989). The

differentiated Sertoli cell may act by directly regulating germinal cell development through the production of growth factors. Contact inhibition or the production of growth inhibitors by the Sertoli cell may also influence germ cell proliferation. Initial experiments with the co-culture of both germinal and Sertoli cells demonstrated an enhanced spermatogenic cell survival and increased germinal cell DNA and RNA synthesis (Rivarola et al (1985). This response is increased in the presence of FSH. These observations imply that the Sertoli cell may produce factors which stimulate germinal cell growth. The complex growth, development and differentiation of these cells will likely require a variety of different growth factors (see Table 1).

Insulin-like growth factor

The insulin-like growth factors (IGFs) derive their name from their structural similarity to insulin (Froesch et al, 1985). IGF-I (previously termed somatomedin C) is considered an essential factor for cellular replication and metabolism. IGF-I appears to be a progression factor for cell growth and regulates DNA synthesis. Liver production and secretion of IGF-I accounts for the high levels of IGF-I in serum and interstitial fluid (Daughaday and Rotwein, 1989). IGF-II, another member of this family, may also act as a growth factor during fetal development.

IGF-I may be required for DNA synthesis and cell division during testicular development and spermatogenesis. IGF-I mRNA was originally identified in whole testis (Casella et al, 1987) and subsequently Sertoli cells have been shown to express and produce this factor (Chatelain et al, 1987; Smith et al, 1987; Closset et al, 1989). Both Sertoli and germinal cells contain receptors for IGF-I (Handelsman et al, 1985; Hansson et al, 1989; Oonk et al, 1988; Vannelli et al, 1988). IGF-I stimulates DNA synthesis (Borland et al, 1984; B. P. Mullaney and M. K. Skinner, unpublished results) as well as increases transferrin and lactate production in immature Sertoli cells (Skinner and Griswold, 1983; Oonk et al, 1989). The presence of the blood–testis barrier prevents interstitial fluid-derived IGF-I from directly affecting sequestered germ cells. Thus, Sertoli cell production of this essential factor may allow for paracrine control of germinal cell proliferation. This is further suggested by the presence of IGF-I receptors and immunoreactivity in spermatocytes and spermatids (Tres et al, 1986). Sertoli cells also appear to produce an IGF-I-binding protein, which may act as a mechanism to concentrate local levels of this factor (Cailleau et al, 1990). Another member of the IGF family, IGF-II, has also been suggested to be involved in local interactions. Although both Sertoli and germinal cells contain IGF-II receptors (O'Brien et al, 1989), IGF-II does not appear to be expressed locally (Murphy et al, 1987). IGF-II appears to stimulate Sertoli cell differentiation, perhaps by cross-reacting with IGF-I receptors (Borland, 1984). Neither IGF-I nor IGF-II have been demonstrated to act directly on germ cells and further study is necessary to understand the physiological importance of these factors.

Nerve growth factor

The neutrotrophic factor β nerve growth factor (β-NGF) is another mitogen which may mediate intercellular interactions involving growth (Yanker and Shooter, 1982). NGF is important for the development and maintenance of sympathetic neurones in the peripheral nervous system and cholinergic neurones in the central nervous system. In other tissues NGF expression typically correlates with the amount of sympathetic innervation. Surprisingly, NGF is expressed at higher levels than expected in testosterone-dependent organs, including the testis.

β-NGF provides an interesting example of a potential germ cell–Sertoli cell interaction. NGF mRNA is specifically expressed in spermatocytes and early spermatids of the adult mouse (Olson et al, 1987; Ayer-LeLievre et al, 1988), while Sertoli cells express NGF receptor (Persson et al, 1990). Hypophysectomy increases NGF receptor mRNA in whole testis and luteinizing hormone (LH) but not FSH replacement returns expression to basal levels. This observation suggests that testosterone down-regulates the receptor and may be an example of a negatively regulated androgen-dependent gene product. However, the physiological significance of NGF-mediated germ cell–Sertoli cell interactions is unclear, neither germinal cell secretion of NGF protein nor Sertoli cell membrane-bound NGF receptors have been demonstrated. Interestingly, levels of NGF receptor may also correlate with stage VI–VIII of the seminiferous cycle, perhaps stimulating the Sertoli cell for later steps in germ cell maturation. The function of NGF in the testis is not known and requires further study.

Testicular interleukin-1

The interleukins (ILs) are a family of cytokines produced by activated lymphocytes and macrophages. One of these factors, IL-1, may play an important role in mediating cellular activation during inflammation and infection (Durum et al, 1985). The β form of IL-1 is typically secreted by lymphocytes; however, IL-1α is produced by non-immune tissues. The mitogenic properties of these factors suggest that IL-1 may mediate growth regulation.

The testis also appears to be a site of cytokine production and includes the testicular IL-1-like factor. IL-1α-like activity was isolated from cultures of mature Sertoli cells, while being absent from cultures of other testicular cell types (Gustafsson et al, 1988; Khan et al, 1988). IL-1 activity in conditioned media increases at puberty, coinciding with the onset of spermatogenesis (Syed et al, 1988). IL-1 is mitogenic for a variety of cell types, thus the Sertoli cell might directly stimulate germ cell development through production of IL-1. One study indicates that intratesticular injection of IL-1 into hypophysectomized rats stimulates [^{3}H]thymidine incorporation in spermatogonia (Pollanen et al, 1989). Presently, however, it is not known which cells contain IL-1 receptors. Another potential role for this cytokine may be to mediate immune suppression. Bioassay and immunological approaches have been utilized in these studies, but further molecular studies are necessary to clarify these interactions.

Transforming growth factor α

Transforming growth factor α (TGF-α) is one of the structurally related peptides belonging to the epidermal growth factor (EGF) family (Derynck, 1988; Carpenter and Cohen, 1990). Due to similar protein structure, these factors act at the same receptor to stimulate cell growth (Carpenter, 1987). TGF-α is synthesized as a transmembrane precursor, which may activate EGF receptors on neighbouring cells or be proteolitically cleaved, releasing mature peptide. TGF-α was initially identified in neoplastic and developing tissue. Recently, however, TGF-α appears to be produced by non-transformed cells, including tissues requiring active cell proliferation. Thus, TGF-α may play an important role as a growth regulator in normal tissues.

EGF has been implicated in the maintenance of spermatogenesis (Tsutsumi et al, 1986; Stastny and Cohen, 1972). Sialoadenectomized mice show 50% reduction of mature sperm, while EGF replacement returns spermatogenesis to normal levels. However, circulating concentrations of EGF are considered too low to mediate endocrine action (Carpenter and Zendegui, 1986). EGF does not appear to be expressed in the testis (Skinner et al, 1989) and these effects may be mediated by a locally produced EGF-like factor. Other studies support this idea, including a report that Sertoli cells secrete a factor that blocks EGF from binding to its receptor (Holmes et al, 1986). TGF-α is an EGF-like factor which may mediate these effects. Sertoli cells but not germinal cells express the gene for TGF-α and produce this factor (Skinner et al, 1989). At present it is unclear whether Sertoli or germinal cells contain receptors for this peptide. Scatchard analysis and histochemistry do not indicate the presence of receptors on differentiated Sertoli cells (Skinner et al, 1989; Stubbs et al, 1990). However, another report presents immunological evidence that Sertoli cells may contain EGF receptors (Suarez-Quian et al, 1989). These differences might be explained by limitations in the sensitivity of binding analysis, antibody cross-reactivity or possible expression of a non-functional truncated form of the receptor. Further examination utilizing molecular probes for the receptor are necessary. The role of TGF-α in Sertoli cell–germ cell interactions is not clear. Speculation that developing spermatogonia respond to TGF-α might provide an appropriate mechanism for Sertoli cells to influence spermatogonial growth.

Transforming growth factor β

Transforming growth factor β (TGF-β) is a multifunctional regulatory molecule which can stimulate or inhibit aspects of cellular growth and differentiation (Roberts and Sporn, 1988). In general, TGF-β acts as a growth inhibitor, specifically inhibiting EGF/TGF-α-stimulated cell proliferation. TGF-β can also promote cellular differentiation, extracellular matrix production and chemotaxis. Different types of TGF-β are produced as latent secreted precursors. Most cell types contain receptors for this ubiquitous factor, thus local activation of latent precursor may be important in regulating cell–cell interactions. Due to the diverse actions of TGF-β, it is likely that this factor will be important in co-ordinating tissue development and function.

TGF-β may act as a multifunctional agent in the seminiferous tubule. Growth inhibitors may be necessary to prevent spermatogonial growth before puberty and to terminate growth of the maturing Sertoli cell. Studies suggest that TGF-β is produced by Sertoli cells and may be modulated by gonadotrophins (Skinner and Moses, 1989; Benahmed et al, 1988). Northern analysis indicates that Sertoli cells express both TGF-β1 and TGF-β3 (Skinner and Moses, 1989; B. P. Mullaney and M. K. Skinner, unpublished results). Interestingly, the testis appears to be one of the few tissues where TGF-β3 is expressed (Miller et al, 1989). These molecules may act in a similar fashion but may be under different hormonal regulation, as indicated by their different upstream gene regulatory regions. TGF-β does not appear to dramatically affect immature Sertoli cell growth or cellular differentiation (Skinner and Moses, 1989). However, TGF-β may be important in regulating environmental interactions necessary for spermatogenesis. TGF-β decreases Sertoli cell plasminogen activator production, perhaps involved in tissue remodelling for germ cell development (Nargolwalla et al, 1990). Due to the antagonistic growth regulation of TGF-α by TGF-β, the local production of TGF-β may act to limit TGF-α action in the tubule.

Fibroblast growth factor

Fibroblast growth factors (FGFs) can influence aspects of both cellular growth and differentiation (Gospodarowicz et al, 1987). FGF has an affinity for heparin found in extracellular matrix accounting for the high potency of this mitogen. Aside from growth stimulation, recent studies indicate that FGF may play a critical role in angiogenesis and tissue repair. The many cellular targets and widespread tissue distribution of FGF suggest that these growth factors may mediate effects in many organ systems including the testis (Gospodarowicz and Ferrara, 1989).

Basic FGF (bFGF) has been isolated from bovine and human testis (Ueno et al, 1987; Story et al, 1988). Sertoli cells appear to produce this factor, although localization of FGF expression has not been demonstrated (Smith et al, 1989). The angiogenic properties of FGF suggest that this factor may be involved in vascularization of this tissue during development. FGF is mitogenic for immature Sertoli cells (Jaillard et al, 1987; Smith et al, 1989). bFGF may also be important in tissue remodelling for spermatogenesis in its ability to stimulate Sertoli cell plasminogen activator activity (Jaillard et al, 1987). FGF action on germ cells has not been demonstrated and further molecular studies are necessary to localize cellular expression of FGF and determine its role in specific testicular cell–cell interactions.

Seminiferous growth factor and Sertoli cell-secreted growth factor

Other mitogenic factors have been implicated in Sertoli cell–germinal cell interactions including seminiferous growth factor (SGF) and Sertoli cell-secreted growth factor (SCSGF). SGF was the first identified mitogenic factor in the tubule (Feig et al, 1980). This 16 kDa mitogen was isolated from

Sertoli cell cultures based on its affinity for heparin and appears immunologically distinct from FGF (Feig et al, 1983; Bellve and Zheng, 1989). SGF stimulates growth in transformed TM4 Sertoli, TM3 Leydig cells and 6-day-old mouse Sertoli cells (Bellve and Feig, 1984). SGF activity has been detected in many species and is predominant during prepubertal development (Feig et al, 1980). Further molecular characterization of this mitogen is necessary. SCSGF has been partially purified and appears mitogenic for a number of cell lines (Buch et al, 1988). SCSGF has some similarities to TGF-α, including its apparent molecular weight of 8 kDa and its ability to displace radiolabelled EGF from binding its receptor (Buch et al, 1988). However, SCSGF stimulates A-431 cell growth, while EGF/TGF-α typically inhibit growth of this cell line. Both SGF and SCSGF have not been fully characterized, and whether these factors are previously identified growth factors remains to be thoroughly investigated. While both factors have mitogenic properties on somatic cells, neither SGF nor SCSGF have been demonstrated to stimulate germinal cell growth.

Endocrine agents

Endocrine agents, like gonadotrophins, communicate signals between different organs and act to alter local cell–cell interactions. Indirectly, endocrine agents may affect different tissues through the production of locally produced growth factors. For example, in the mammary gland oestrogen may stimulate cell growth by increasing TGF-α and decreasing TGF-β production. In the testis, FSH is required for tissue development and differentiation. FSH appears to stimulate immature Sertoli cell growth. Most growth factors (EGF, FGF, IGF) act through mechanisms involving receptor–kinases to initiate the cell growth cycle. In contrast FSH stimulates production of cAMP, usually associated with cellular differentiation rather than growth. Due to these differences in pharmacological mechanism, FSH may not act as a classic direct growth stimulator. Most growth factors directly stimulate DNA synthesis within 18–30 h after treatment; however, FSH stimulates [^{3}H]thymidine incorporation in cultured immature Sertoli cells with maximal effects after 72–96 h (Griswold et al, 1976; Orth and Boehm, 1990). This delayed response further suggests that FSH action may be mediated by stimulation of growth factor production which results in autocrine growth stimulation. Interestingly, gonadotrophin-induced growth may be inhibited by β-endorphin (Orth, 1986; Orth and Boehm, 1990). Thus, local opioid production may allow for modulation of FSH action. Presently, it is not clear which endocrine-regulated growth factors might be responsible for Sertoli or germinal cell growth.

PERITUBULAR CELL–SERTOLI CELL INTERACTIONS

Interactions between the peritubular–myoid cell and the Sertoli cell are postulated to be important for testis function (Skinner, 1987). Growth control is necessary for both cell types during development. Sertoli cells grow

prepubertally, then terminally differentiate and growth is arrested at the onset of puberty. Peritubular cells continuously have a slow proliferation. Growth factor interactions appear to occur between these cell types, which may regulate cellular growth and differentiation and represent a mesenchymal–epithelial cell interaction. Co-culture experiments indicate that the presence of peritubular cells can alter Sertoli cell morphology and enhance Sertoli cell transferrin production (Holmes et al, 1984).

Transforming growth factor α

TGF-α is expressed and produced by both peritubular and Sertoli cells (Skinner et al, 1989). It is unclear whether Sertoli cells express EGF receptors; however, Scatchard analysis indicates that high-affinity EGF receptors are present on peritubular cells (Skinner et al, 1989). TGF-α stimulates peritubular cell DNA synthesis and cell division (Skinner et al, 1989). Both peritubular and Sertoli cell production of TGF-α may contribute to peritubular cell growth. Recent literature suggests EGF may alter Sertoli cell function, including stimulation of lactate and inhibin production (Mallea et al, 1986; Welsh and Hsueh, 1982). Due to potential peritubular–Sertoli interactions, analysis of Sertoli cell function requires pure preparations of Sertoli cells. Some actions of TGF-α/EGF may be mediated indirectly by peritubular cell production of other factors. For example, EGF can stimulate transferrin production in Sertoli–peritubular co-cultures, although in pure preparations of Sertoli cells EGF does not appear to influence transferrin production (Skinner et al, 1989). Two models utilizing TGF-α transgenic mice, which overexpress TGF-α in the testis, show no abnormal features in this tissue (Jhappan et al, 1990; Matsui et al, 1990; B. P. Mullaney and M. K. Skinner, unpublished results). Thus the in vivo role of TGF-α presently remains unclear.

Transforming growth factor β

Both peritubular and Sertoli cells also express and produce TGF-β (Skinner and Moses, 1989). TGF-β acts as a growth inhibitor for peritubular cells and blocks TGF-α-induced peritubular proliferation (B. P. Mullaney and M. K. Skinner, unpublished results). TGF-β has little effect on immature Sertoli cell growth or differentiation (Skinner and Moses, 1989). A number of observations suggest that TGF-β may be important in peritubular cell differentiation. TGF-β may regulate the production of extracellular matrix components by peritubular cells (Skinner and Moses, 1989), and increase production of plasminogen activator inhibitor type 1 (PAI-1) by peritubular cells. TGF-β increases peritubular contractility potentially required for sperm transport in the tubule (Ailenberg et al, 1990) and induces migration and colony formation of peritubular cells in culture (Skinner and Moses, 1989). TGF-β-stimulated chemotaxis may be a mechanism to recruit non-differentiated fibroblasts to the exterior of the tubule during development. Therefore, TGF-β may influence morphogenesis and structural formation of the seminiferous tubule and maintain essential interactions necessary for spermatogenesis.

Insulin-like growth factor I

IGF-I is produced by both peritubular and Sertoli cells (Smith et al, 1987; Cailleau et al, 1990). IGF-I stimulates DNA synthesis in both cell types (Borland et al, 1984; B. P. Mullaney and M. K. Skinner, unpublished results) and stimulates Sertoli cell transferrin and lactate production (Skinner and Griswold, 1983; Oonk et al, 1989). Certain Sertoli genes like inhibin are not regulated by IGF-I (Toebosch et al, 1988). Interstitial fluid contains high amounts of IGF-I readily available to both Sertoli and peritubular cells. Thus the local production of IGF-I may not contribute significantly to autocrine or paracrine interactions involving these somatic cells.

LEYDIG CELL–SERTOLI CELL INTERACTIONS

Leydig cell–Sertoli cell interactions were one of the first testicular cell–cell interactions to be investigated. LH regulates androgen production by Leydig cells that subsequently acts on Sertoli cells to maintain testis function. Androgen levels in vivo, however, are present in excess of those required to maintain spermatogenesis (Santuli et al, 1990). Transient modulation of androgen production by locally produced factors, therefore, may not alter spermatogenesis. The significance of growth factor-mediated Sertoli cell–Leydig cell interactions is unclear.

Insulin-like growth factor I

IGF-I is produced by both Leydig and Sertoli cells (Naville et al, 1990). IGF-I is the only mitogen presently identified to be produced by Leydig cells. IGF-I stimulates Leydig cell steroidogenesis (Kasson and Hsueh, 1987; Perrard-Sapori et al, 1987). LH upregulates IGF-I receptors on Leydig cells (Lin et al, 1986; Kasson and Hsueh, 1987; Lin et al, 1987b). Gonadotrophin stimulation also increases IGF-I-binding protein production by both cell types, perhaps allowing for local modulation of IGF-I levels (Cailleau et al, 1990). Both somatic cell types, however, are exposed to high levels of IGF-I present in interstitial fluid. IGF-I may influence cell function, but speculated cell–cell interactions involving IGF-I need to be questioned.

Transforming growth factors α and β

Interstitial cells contain EGF receptors but do not proliferate in response to EGF (Ascoli, 1981). EGF/TGF-α also inhibits LH-induced steroidogenesis and decreases LH receptor binding (Welsh and Hseuh, 1982). Sertoli production of TGF-α may regulate interstitial cell growth during development. Recently, TGF-α has been immunohistochemically detected in Leydig cells (Teerds et al, 1990). Leydig cell production of TGF-α has not been demonstrated and this observation may be due to membrane-bound TGF-α precursor or endocytosis of paracrine-derived TGF-α. Further

investigation is needed to determine the importance of Sertoli-derived TGF-α for Leydig cell growth.

Sertoli cell production of TGF-β may also regulate Leydig cell growth and differentiation. Similar to TGF-α, TGF-β inhibits LH-induced steroidogenesis, possibly by decreasing LH receptor binding (Avallet et al, 1987; Lin et al, 1987a). During development, the growth of maturing Leydig cells slows and may require a growth inhibitor like TGF-β. TGF-β decreases DNA synthesis in a transformed Leydig cell line; however, TGF-β has little effect on Leydig cell growth in primary culture (Benhamed et al, 1989; Gonzalez-Manchon and Vale, 1989). The local production of TGF-β in the interstitium needs to be determined to elucidate the importance of TGF-β-mediated Sertoli cell–Leydig cell interactions.

Fibroblast growth factor and interleukin 1

Other Sertoli cell-produced growth factors may be involved in Sertoli–Leydig interactions including FGF and IL-1. The mitogenic actions of these factors on Leydig cell growth have not been determined. However, FGF is reported both to stimulate and inhibit Leydig steroidogenesis in different species (Fauser et al, 1988; Raeside et al, 1988; Sordoillet et al, 1988; Murono and Washburn, 1990a). IL-1 also inhibits Leydig steroidogenesis (Calkins et al, 1988). Thus, the predominate action of growth factors on Leydig cell differentiation is inhibitory.

LEYDIG CELL–PERITUBULAR CELL INTERACTIONS

Growth factors may mediate interactions between Leydig and peritubular cells, although the major regulatory interaction between these cells involves androgens, which are needed for peritubular differentiation (Hovatta, 1972). In the mature testis both these cells require continuous but slow growth regulation. The peritubular cell and Sertoli cell produce similar growth factors, thus proposed Leydig–peritubular interactions may be similar to Leydig–Sertoli interactions.

Insulin-like growth factor I

Both Leydig and peritubular cells produce IGF-I, which has been suggested to be involved in intercellular communication. LH stimulates Leydig IGF-I production, but androgens do not appear to modulate peritubular IGF-I production (Cailleau et al, 1990). In comparison to the Leydig cell, peritubular cells appear to produce relatively low amounts of IGF-I-binding proteins (Cailleau et al, 1990). The physiological significance of these observations are unclear since both of these somatic cell types have ready access to interstitial fluid containing a high concentration of IGF-I.

Transforming growth factors α and β

Leydig cell proliferation may be in part regulated by production of TGF-α

and TGF-β by peritubular cells. A paracrine interaction involving TGF-α may account for TGF-α immunoreactivity detected in Leydig cells (Teerds et al, 1990). TGF-β may act as a growth inhibitor to limit TGF-α-induced proliferation. Both of these factors appear to inhibit Leydig steroidogenesis; however, as mentioned previously, transient alterations in androgen production may not affect spermatogenesis. Further identification of growth factors produced by the interstitial cell and better biochemical markers of peritubular differentiation are necessary to examine Leydig–peritubular interactions.

ADDITIONAL CELL–CELL INTERACTIONS

A number of other somatic cell types are present in the testis and may contribute to local cell–cell interactions involving growth factors. Stromal fibroblasts contribute to the interstitial cell population. The continuous growth and defined turnover of Leydig and peritubular cells may require stem cell precursors from this stromal population. The LH and testosterone-dependent growth of the precursors (Hardy et al, 1990) may be mediated by locally produced growth factors. A majority of the remaining interstitial cell population includes testicular macrophages. Sertoli cell production of IL-1 may stimulate growth and activate these cells to co-ordinate immune suppression in the tubule. These immune cells also produce a number of cytokines which may influence local cell growth and function. The high vascularization of the testis suggests that vascular and lymphatic endothelial cells may be involved in local interactions. Endothelial cells are known to produce a number of regulatory factors including platelet-derived growth factor (PDGF), which has been noted to inhibit Leydig steroidogenesis (Murono and Washburn, 1990b). Sertoli cell production of FGF may also regulate angiogenesis during testis development. Thus, a variety of somatic cell types may contribute to growth factor-mediated interactions in the testis, although few of these interactions have been characterized.

CONCLUSIONS

Cellular differentiation versus growth

While control of growth is necessary for testis cell proliferation, control of differentiation is vital for development of specialized cellular functions and may require specific non-mitogenic 'differentiation' factors. Differentiation and growth appear to be distinct processes that require a complex activation of a specific set of genes. The dissimilar nature of these two events suggests that both growth and differentiation may not occur simultaneously in a normal cell. A developing hypothesis is that the control of growth and differentiation may be inversely related. Growth factors directly alter the cell growth cycle to promote a less differentiated growth state. While growth

stimulators such as TGF-α or FGF promote cell proliferation and indirectly inhibit differentiation, growth inhibitors such as TGF-β can promote cellular differentiation. However, TGF-β has little positive effect on Leydig or Sertoli cell differentiation, suggesting that other differentiation factors may be required for testis function. An example of a potential testicular differentiation factor for Sertoli cells has been identified, and termed PMODS (Skinner et al, 1987). Peritubular cells produce this factor which modulates cultured Sertoli cell function to a greater extent than other known hormones, including FSH (Norton and Skinner, 1989). As a differentiation factor, PMODS stimulates cellular function without affecting cellular growth. Thus, the local production of both growth factors and differentiation factors may be required for organ development and function.

Testicular neoplasia

Pertubation of regulatory factors controlling normal tissue growth and differentiation may result in neoplasia. Growth factors have been implicated in carcinogenesis and may be potentially involved in the development of testicular neoplasms. Due to the rapid proliferation of germinal cells during spermatogenesis, it is not surprising that approximately 95% of malignant testicular neoplasms are of germinal cell origin. These tumours are one of the most common forms of cancer in young adult males and include seminomas, teratomas and embryonal cell carcinomas (Javadpour, 1986; Mostofi et al, 1990). Many of these neoplasms are associated with cryptorchidism, abnormal descension and development of the testis. In the adult the terminal differentiation of the Sertoli cell probably accounts for the rare occurrence of tumours of Sertoli origin. Many mechanisms involving growth factors identified in the testis may account for transformation of normal cells. Alterations in expression and production of growth factors may lead to abnormal growth. For example, overexpression of growth stimulators like TGF-α or decreases in growth inhibitors like TGF-β may lead to enhanced cell growth. Alterations in activation of growth factor precursors, changes in growth factor receptor populations or lack of proper differentiation factors could also contribute to transformation. The metastatic potential of these neoplasms may also be influenced by TGF-β and FGF which can alter matrix proteases enhancing tumour invasion. FGF-type angiogenic factors may also influence vascularization required for rapidly developing tumours. At present very little is known about the molecular mechanisms resulting in testicular cancer; however, abnormal regulation of locally produced growth factors may be involved.

SUMMARY

The development of testicular function may require local cell–cell interactions to regulate tissue growth and differentiation. Locally produced growth factors may mediate the differential growth of mesenchymal, epithelial and germinal cells that occurs during fetal, prepubertal and

Table 2. Potential growth factor interactions in the testis.

Growth factor	Proposed cellular source	Expression	localization	secretion	Proposed cellular action			
					Leydig	Peritubular	Sertoli	Germinal
IGF-I	LC	×	×	×	+ Steroidogenesis	+ Growth	+ Growth + Differentiation	+ Growth(?)
	PC		×	×				
	SC		×	×				
	GC		×					
TGF-α	PC	×	×	×	+ Growth(?)	+ Growth	+ Differentiation(?)	?
	SC	×		×	− Steroidogenesis			
	LC		×					
TGF-β	PC	×		×	− Growth	− Growth	Minimal effects	?
	SC	×		×	− Steroidogenesis	+ Differentiation		
IL-1	SC			×	− Steroidogenesis	?	?	+ Growth(?)
β-NGF	GC	×	×		?	?	?	?
FGF	SC			×	± Steroidogenesis	?	+ Growth	?
SGF	SC			×	?	?	+ Growth	?
SCSGF	SC			×	?	?	?	?

GC, germinal cell; LC, Leydig cell; PC, peritubular cell; SC, Sertoli cell.

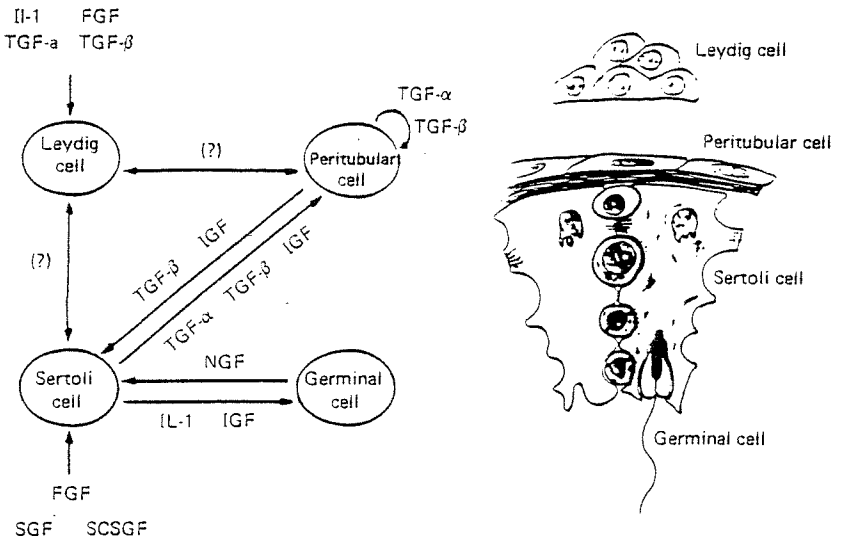

Figure 1. Proposed growth factor interactions in the testis.

postpubertal testis development. The complex co-ordination of differential and temporal cellular growth suggests that a variety of locally produced factors may be involved. Presently, a number of growth factors have been identified in the testis, including IGF-I, TGF-α, TGF-β, NGF, IL-1, FGF, SGF and SCSGF. These factors may mediate interactions involving growth stimulation, growth inhibition and differentiation in this tissue (Table 2 and Figure 1). Endocrine agents are also necessary for testis development and function. In many organs, endocrine hormones appear to alter local cell–cell interactions. Similarly, gonadotrophins may modulate growth factor interactions within the testis. Understanding testicular cell-cell interactions involving growth factors requires evaluation of the cellular site of factor expression, production, secretion, target cell action and in vivo significance. Presently, none of the proposed cell–cell interactions involving growth factors have evaluated all these criteria. Further cellular and molecular analysis of these intercellular interactions are necessary to clarify the role of growth factors in the development and maintenance of testicular function.

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