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Review

# Growth Factors and the Epididymis

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## *Mechanisms of Cell Communication*

Mechanisms of cell communication are of paramount importance in multicellular organisms, because they are responsible for the coordination of basic processes, such as growth, differentiation, immunity, motility, and transport. The most common mechanism of cell communication is that mediated by extracellular signaling molecules that bind membrane receptors of target cells and activate a host of intracellular signaling cascades that lead to specific biological responses. Signaling is classified as endocrine or paracrine on the basis of the proximity of the target cell. In endocrine signaling, target cells are located in a distant site and are influenced by signaling molecules arriving via the vasculature. In paracrine signaling, target cells are influenced by the secretion of neighboring cells. Autocrine and juxtacrine modes of secretion have also been recognized. During autocrine secretion, signaling molecules act on the cell that secreted them, and during juxtacrine secretion, plasma membrane molecules of one cell activate receptors in the membrane of a neighboring cell. In the epididymis, a "lumicrine" mechanism must also be considered in which signaling molecules reach the epididymis from another organ, the testis, via the luminal compartment, rather than the vasculature (Turner and Miller, 1997; Hinton et al, 1998). In recent years, testicular factors and lumicrine signaling in general have been recognized as important contributors to the physiology of the epididymis (Hinton et al, 2003).

## *Signaling by Growth Factors*

Growth factors are a group of polypeptides that promote cell division and differentiation, usually in a paracrine or endocrine fashion. They are grouped into families on the basis of their structure, with most of the families containing several members. They act by activating cell membrane receptors

with tyrosine kinase (RTK) activity or, less frequently, receptors with serine/threonine kinase activity. The mechanism of RTK activation following binding of a growth factor involves dimerization and autophosphorylation of tyrosine residues, which induces the receptor to trigger the activation of mitogen-activated protein kinases (MAPKs) (Schlessinger, 2000). There are 3 well-known groups of MAPKs: extracellular signal-regulated kinases (ERKs), c-Jun amino-terminal kinases (JNKs), and p38 kinases (Johnson and Lapadat, 2002). These are highly conserved enzymes that share the common property of being activated by 3-tier phosphorylation cascades in response to a variety of stimuli. The ERK pathway is preferentially stimulated by growth factors and phorbol esters, whereas the JNK and p38 pathways are usually activated by ionizing radiation, osmotic shock, and other stress stimuli.

RTK stimulation by growth factors is usually associated with activation of ERKs, particularly ERK1 and ERK2. These are highly similar serine/threonine kinases with sizes of 44 and 42 kd, respectively, that are themselves activated by double phosphorylation on tyrosine and threonine residues. The most important intracellular signaling cascade for ERK activation involves the activation of Ras, a membrane-bound GTP-binding protein (Figure 1). Similar to other GTP-binding proteins, Ras is activated by exchanging its bound GDP for GTP. This initiates a cascade of intracellular phosphorylation events in which upstream kinases phosporylate (ie, activate) downstream kinases according to the following sequence: Raf (also known as MEK kinase, MEKK, or MAPKKK), MEK (or MAPKK kinase), and ERK (also known as MAPK; Wilkinson and Millar, 2000). A host of tissue-specific adapters and scaffolds are also required for the activation of this cascade, but will not be discussed here because they are beyond the scope of this review (Roux and Blenis, 2004). Phosphorylated ERKs play a central role in growth factor signaling because they phosphorylate a variety of membrane and cytoskeletal proteins, downstream kinases, and, most importantly, transcription factors, such as Elk-1 and the proto-oncogene product *c-myc*, that ultimately mediate specific cell responses (Schaeffer and Weber, 1999).

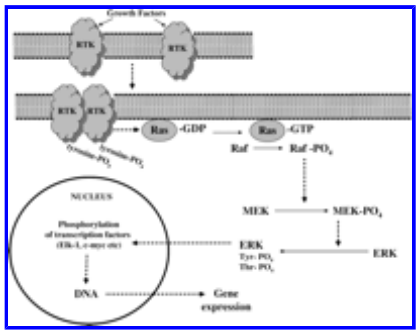


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Figure 1. Intracellular signaling by growth factors. Growth factors mediate their actions by stimulating mitogen-activated protein kinases (MAPKs). These intracellular kinases are activated by phosphorylation and are usually classified into 3 groups: extracellular signal-regulated kinases (ERKs) 1 and 2, c-Jun amino-terminal kinases (JNK), and p38 kinases. The effects of growth factors are generally mediated by stimulation of the ERK1/2 pathway, the central aspects of which are depicted here (see text for references). Growth factors attach to cell membrane receptors known as receptor tyrosine kinases (RTKs), which upon growth factor binding, dimerize and autophosphorylate tyrosine residues. These activated RTKs induce Ras to undergo activation by exchanging its bound GDP for GTP. Activated Ras promotes Raf phosphorylation in the vicinity of the cell membrane. There are actually 3 known Rafs, collectively referred to as "Raf" in the figure. Raf phosphorylates MEK (either MEK1 or MEK2), which are referred to here as "MEK." MEK activates ERK by phosphorylation of tyrosine and threonine residues. There are also 2 known ERKs, ERK1 and ERK2, referred as "ERK" in the figure. Activated ERKs accumulate in the nucleus and induce the phoshorylation of numerous substrates, including transcription factors, which promote the expression of specific genes. (Parts of the Raf-MEK-ERK pathway are also known by a MEK/MAPK terminology [see text].)

## *Growth Factors and the Epididymis*

In recent years, a number of investigators have explored the role of growth factors in the physiology of the epididymis and the male reproductive system in general. Epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF) have received the most attention, but other growth factors have also been studied, and a concise review seems appropriate at this time because more-rapid advances are being made in the field. We have also included here references to research demonstrating the presence of growth factors in the testis, given the fact that substances secreted by the testis may influence the epididymis in a lumicrine fashion. Finally, we illustrate mRNA expression data for selected growth factors and growth factor receptors from the recently published mouse epididymal transcriptome (MET) database (available at: http://www.mrg.genetics.washington.edu), which details gene expression of all mouse epididymal segments (Johnston et al, 2005). In this study, which is a collaboration between our laboratory and those of others, mRNA expression in all 10 segments of the mouse epididymis was analyzed using Affimetrix gene chips (Figure 2).

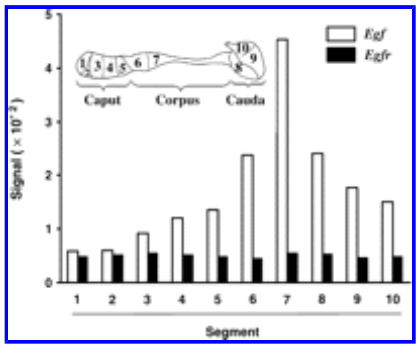


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Figure 2. Expression of *Egf* and *Efgr* in the epididymal segments of the mouse. Expression data were obtained from a microarray analysis originally performed by our laboratory (Johnston et al, 2005). Briefly, epididymides were obtained from anesthetized C57BL/6 mice and segments immediately dissected. Similar segments from 5 animals were pooled, and mRNA was obtained using standard phenol/guanidine isothiocyanate extraction. mRNA was then used as a template to generate biotin-labeled cRNA. Gene expression was evaluated as the hybridization strength of fragmented cRNA to MOE430A and MOE430B Affimetrix gene arrays. The figure shows hybridization signals for *Egf* (open bars) and *Egfr* (black bars) in all 10 segments. Each data point corresponds to the average hybridization signal obtained from 4-8 replicates of the pooled samples described above. Segmentation of the mouse epididymis (inset). Numbers indicate the segment number as used in the main figure, segment 1 being most proximal and segment 10 being most distal.

*EGF*— An early indication of a potential role for EGF in the male reproductive system was the observation that the removal of the submaxillar gland—an organ rich in EGF—led to a marked decrease of epididymal sperm cells in adult mice without affecting the levels of testosterone or follicle-stimulating hormone (Tsutsumi et al, 1986). Moreover, changes were reversed by administration of EGF, confirming that the decrease in epididymal sperm cells was caused by EGF deprivation. It has been reported, however, that this effect is much smaller than originally claimed (Russell et al, 1990). EGF is a 53-amino acid, 6-kd protein that was originally discovered during the observation that mice submaxillary gland extracts accelerate the development of newborn animals (Cohen, 1962; Carpenter and Cohen, 1990). Over the years, EGF has proven to be a growth factor that stimulates not only the proliferation of epithelial cells, but also a number of other cell types of both ectodermic and mesodermic origin. The effect of EGF on spermatogenesis prompted studies that have demonstrated the presence of both EGF (Byyny et al, 1972; Elson et al, 1984) and EGF receptors (EGFRs) (Suarez-Quian et al, 1989; Suarez-Quian and Niklinski, 1990; Foresta et al, 1991) in the testes of different species, including humans, mice, and rats. Moreover, studies in mice have demonstrated the ability of testicular extracts to displace $^{125}$I-EGF from its receptor in a manner indistinguishable from that of submaxillary gland extracts, which suggests the presence of the canonical, 6-kd form of EGF in the testis (Radhakrishnan et al, 1992). Studies involving nonhuman primates confirmed the presence of the EGF receptor in the testis and demonstrated that this receptor is also present in the epididymis and the vas deferens (Radhakrishnan and Suarez-Quian, 1992). The EGFR is located in both the basolateral and apical borders of epididymal epithelial

cells. Moreover, the luminal staining showed vesicular images consistent with a pattern of receptor-mediated endocytosis. Further immunohistochemical studies on C3H mice showed that the intracellular localization of the EGF receptor varies along the length of the epididymis: in the caput, staining was intense and evenly distributed in the cytoplasm of principal cells, whereas in the corpus and cauda, staining was limited to the apical cytoplasm (Suarez-Quian et al, 1994).

The presence of a growth factor in a tissue does not necessarily mean that the growth factor is synthesized there. Other investigations have shown that the testis is, in fact, a source of EGF (Radhakrishnan et al, 1992). In these studies, the presence of the intracellular EGF precursor ($EGF_p$) in the mouse testis was investigated by Western blotting and immunohistochemistry. $EGF_p$ is a 140-kd integral-membrane protein from which the 6-kd, active EGF is released by proteolytic cleavage and whose presence in a given tissue strongly suggest that EGF is locally synthesized. In contrast to EGF, which was found to be widely distributed in the testis, including in Sertoli cells, $EGF_p$ was restricted to germ cells, suggesting a paracrine signaling mechanism. The presence in the testis of EGF, which is usually a paracrine-acting growth factor, is important for epididymal physiology, given the potential lumicrine influence of testicular factors on the epididymis (Fawcett and Hoffer, 1979; Nicander et al, 1983; Hinton et al, 1998; Hinton et al, 2003).

Apart from a single report of putative EGF in the human epididymis (Elson et al, 1984), the presence of EGF in the epididymis has not been determined. In this regard, it is interesting that data from MET database demonstrate that *Egf* is expressed in the epididymis in a segment-specific manner (Figure 2). Thus, it is possible that, in addition to the lumicrine model, cells of the epididymal interstitium (eg, macrophages and fibroblasts) synthesize and secrete EGF to communicate with cells in the adjacent tubule epithelium.

Interestingly, expression of *Egf* is relatively low in the most proximal segments of the caput epididymis, but increases approximately 4-fold by segment 7, which comprises most of the corpus region. In this part of the duct, EGF from the testis may no longer be available to the epithelium, and it can be hypothesized that the epithelial cells may depend more on local, paracrine-acting EGF. It has been reported in the MET databases that expression of the gene encoding the EGFR is relatively constant throughout the epididymis (Figure 2); thus, if the epididymal expression of *Egf* and *Egfr* are confirmed by protein presence, the data will be consistent with the concept that lumicrine EGF is important in the proximal caput of the mouse epididymis, and that paracrine EGF is more important in more distal segments, especially the corpus epididymis. Whether this hypothesis is true in the mouse or other species is yet to be determined, but these initial gene expression data suggest that EGF plays a meaningful role in the segmental regulation of the epididymal tubule.

Of note, the MET database shows that *Cd97*, a member of the EGF 7-span transmembrane receptor family, is up to 10-fold more highly expressed than *Egfr* in the mouse epididymis, especially in the cauda segments (data not shown). CD97 has an extended extracellular region with several N-terminal EGF-like domains that mediate binding to its ligand (Jaspers et al, 2001). The role of CD97 in cell signaling in macrophages and dendritic cells has previously been studied, and it will be interesting to determine whether these cells are playing a regulatory role within the segment(s) in which they reside.

*Fibroblast Growth Factors*— Fibroblast growth factors (FGFs) are a large family (>20 members) of related polypeptides whose function, in addition to promoting cell growth, includes the regulation of cell motility, differentiation, chemotaxis, and apoptosis (Böttcher and Niehrs, 2005). They are found in a wide variety of species and play an important role in the ontogeny of both

vertebrates and invertebrates, although their biological role in adult tissues is much less understood. Vertebrate FGFs are single-chain polypeptides with an approximate size of 17-34 kd that share a common, highly conserved central core domain of 120 amino acids and exhibit preferential binding to specific FGF receptors (FGFRs; Ortniz and Itoh, 2001).

Similar to other growth factor receptors, FGFRs are membrane proteins with extracellular ligand-binding domains and intracellular tyrosine kinase domains that promote autophosphorylation and dimerization. Four different types of FGFRs have been described (FGFR 1-4) that correspond to the products of 4 highly related genes (*Fgfr-1-4*). Alternative splicing of *Fgfr* transcripts, particularly types 1-3, generates a variety of receptor isoforms that are differentially expressed along the mouse epididymis (MET database).

Signaling by FGFs usually proceeds via the classical Ras/ERK cascade (see above), although in some tissues, signaling occurs via activation of PLCγ (Böttcher and Niehrs, 2005). In the latter case, activated FGFRs bind PLCγ, which hydrolyzes phosphatidylinositol-4,5-disphosphate into diacylglycerol and inositol 1,4,5-trisphosphate (IP3). This provides a link to signaling cascades mediated by $Ca^{2+}$, given the $Ca^{2+}$-releasing effect of IP3 on intracellular $Ca^{2+}$ stores.

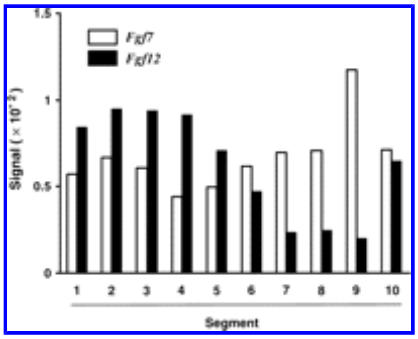


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Figure 3. Expression of *Fgf7* (open bars) and *Fgf12* (black bars) in the epididymal segments of the mouse. Expression data were obtained as described in Figure 2.

The first FGF was discovered as a compound that stimulates the proliferation of NIH3T3 cells (Gospodarowicz and Moran, 1975). It was later dubbed "basic" to be distinguished from acidic FGF extracted from the bovine brain. Similar to EGF, bFGF has been shown to be present in the testis (Ueno et al, 1987; Mayerhofer et al, 1991; Han et al, 1993). Additional studies have shown that the ovine rete testis fluid contains a factor that stimulates the growth of fibroblasts (Brown et al, 1982), and that homogenates of bovine and human epididymides exhibit bFGF-like activity (Story et al, 1988). MET data suggest the occurrence of differential expression of both *Fgf* and *Fgfrs* along the length of the epididymis. The 2 most highly expressed FGF genes, *Fgf7* and *Fgf12*, show differential pattern of expression, with *Fgf12* being more highly expressed in caput segments and *Fgf7* peaking in segment 9 of the cauda (Figure 3). Among the FGFR genes, *Fgfr2* is the most abundant by far and peaks in the cauda epididymis (Figure 4).

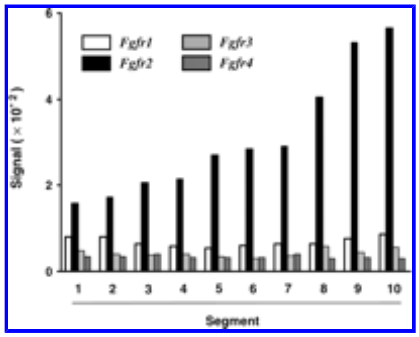


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Figure 4. Expression of *Fgfr1* (open bars), *Fgfr2* (black bars), *Fgfr3* (light grey bars), and *Fgfr4* (dark grey bars) in the epididymal segments of the mouse. Expression data were obtained as described in Figure 2.

Although the biological relevance of these findings is not yet understood, results reported by Hinton et al (2003) suggest a lumicrine mode of action for FGF. Hinton and associates investigated the influence of bFGF on the activity of epididymal γ-glutamyl transpeptidase (GGT) after efferent duct ligation, which deprives the epididymal lumen of testicular factors (Lan et al, 1998). GGT is believed to help protect sperm cells from oxidative stress by maintaining adequate levels of glutathione, which prevents the oxidation of protein thiols to disulfides. Lan et al (1998) showed that bFGF treatment restores the reduced levels of GGT and GGT activity that are observed in the initial segment of the rat epididymis after efferent duct ligation, and concluded that low GGT activity after efferent duct ligation reflects the absence of stimulation by a testicular factor or factors. Interestingly, GGT activity can be restored with rete testis fluid, but not with EGF. Taken together, these experiments provide an example of the regulation of an important epididymal function by testicular factors, and suggest bFGF as a likely mediator. In a related study, the presence of FGFRs in cells from the initial segment of the rat epididymis was investigated by RT-PCR and immunoblotting (Kirby et al, 2003). All 4 types of FGFRs were found to be expressed in the rat initial segment, with principal cells only expressing FGFR-1 (splice variant IIIc). Once again, these results suggest a trophic influence of the testicle on the epididymis.

*VEGF*— VEGF was initially characterized as a factor that increases the permeability of tumor blood vessels (Senger et al, 1983) and was subsequently found to be a potent mitogen, specific for vascular endothelial cells (Leung et al, 1989; Keck et al, 1989). VEGF plays a key role in angiogenesis during embryogenesis and cancer pathogenesis because tumor development requires the formation of new blood and lymphatic vessels (Hicklin and Ellis, 2005). During the past few years, a plethora of new biological roles for VEGF have been proposed, mostly in relation to the genesis and progression of tumors. Some of these roles are immunosuppression, regulation of hematopoiesis, and suppression of apoptosis (Xie et al, 2004).

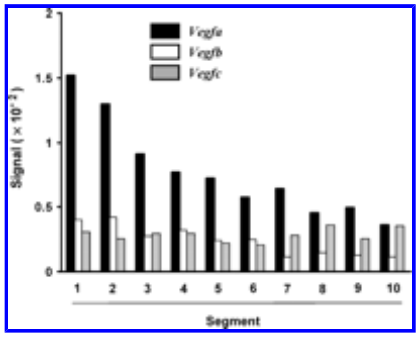


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Figure 5. Expression of *Vegfa* (black bars), *Vegfb* (open bars), and *Vegfc* (grey bars) in the epididymal segments of the mouse. Expression data were obtained as described in Figure 2.

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The VEGF family comprises 6 members: VEGF A, B, C, D, and E; and placenta growth factor. The most commonly expressed member is VEGF-A, a 165-amino acid, 45-kd homodimeric glycoprotein usually referred to as "VEGF." In addition, *Vegfa* can be alternatively spliced to generate isoforms with 121, 189, 206, and, more rarely, 145 and 183 amino acids. *Vegfa* is the most abundantly expressed *Vegf* in the mouse epididymis (Figure 5), especially in the proximal segments. Members of the VEGF family interact with different affinities with 3 different RTKs: VEGFR-1, VEGFR-2, and VEGFR-3 (Ferrara et al, 2003). These receptors are also known as Flt-1, KDR (or Flk-1 for the murine type), and Flt-4, respectively. Of these, the gene encoding VEGFR-2 is expressed more than 2-fold more times than the other forms in the mouse epididymis, whereas the gene encoding VEGFR-1 has the lowest expression (MET database).

The expressions of VEGF and its receptors, as well as the effect of VEGF on epididymal capillaries, have been recently investigated in the human epididymis (Ergün et al, 1998). RT-PCR analysis has shown that VEGF mRNA, as well as mRNA for the VEGF receptors Flt-1 and KDR, are present in fresh human epididymis homogenates. The expression of VEGF protein was confirmed by immunoblotting with polyclonal anti-VEGF antibodies. Furthermore, VEGF and its receptors were localized by immunohistochemistry in Bouin-fixed sections of human tissue samples. VEGF was mainly localized to peritubular myoid cells and absent in endothelial cells from blood vessels. KDR was observed in the capillary vascular endothelium and in some basal cells. Staining for Flt-1 in the human epididymis was mostly negative, with the exception of some reactivity in interstitial lymphatic vessels, but was strongly positive in basal and ciliated cells of the distal part of the efferent ducts.

In the mouse, Flt-1 has been detected by in situ hybridization in endothelial cells of interstitial blood vessels throughout the epididymis (Korpelainen et al, 1998). Finally, electron microscopy of human epididymides treated topically with VEGF has shown an increased number of fenestrated capillaries, opened tight junctions, and intracellular vesicles, which suggests that several aspects of epididymal biology may be influenced by VEGF via activation of the VEGF receptor.

A potential role of VEGF in the reproductive system of the mouse has been explored in transgenic mice expressing VEGF under the mouse mammary tumor virus LTR promoter (Korpelainen et al, 1998). These mice show aberrant spermatogenesis and a malformed epididymis and, as a consequence, are not fertile. The epididymides of these animals are enlarged and swollen. Histological examination has shown, among other changes, that the epithelium in the caput epididymis is reduced in height, compared with wild-type controls, whereas the cauda shows a hyperplastic epithelium. These characteristics are typical of those seen in mice with a chronic, proximal duct obstruction, although this was not verified in the study by Korpelainen et al (1998). Interestingly, the areas of epithelial hyperproliferation exhibited higher expression of both VEGF and VEGFR, as well as an increased number of blood vessels, and increased capillary permeability (Korpelainen et al, 1998). Whether this reflects on the role of VEGF in the epididymis, or merely on a related aspect of the phenotype remains unknown.

Immunohistochemical studies with an antibody directed against the amino terminal portion of VEGF show that VEGF immunoreactivity in the testes is localized to Leydig and Sertoli cells (Ergün et al, 1997). Secretion from Sertoli cells could potentially reach the epididymis via the lumicrine route and influence the activity of the epididymal epithelium. This would be consistent with the existing

evidence that testicular factors, potentially including VEGF, play an important role in the physiology of the epididymis. Alternatively, it may be the case that VEGF could be playing a paracrine role in the early segments of the mouse epididymis because VEGF expression is relatively high in this region (Figure 5). This would be consistent with the findings of Ergün et al (1998) that showed immunolocalization of VEGF to cells in the human epididymal interstitial compartment, including macrophages.

*Other Growth Factors*— Nerve growth factor (NGF) is a disulfide-linked homodimer of two 13.5-kd monomers that plays a central role in the development of sympathetic neurons and also promotes growth and differentiation (Chao, 2003). It is also known as "ß-NGF," because mature NGF constitutes the ß chain of a 140-kd precursor. NGF has been detected in the mouse and rat testis and epididymis by in situ hybridization and Northern blotting (Ayer-LeLievre et al, 1988). In the adult mouse epididymis, strong in situ hybridization signals were observed in the epithelium of the corpus and in some principal cells of the cauda. The NGF receptor, however, although abundant in the mouse testis is almost absent in the epididymis, as revealed by in situ hybridization. This is also consistent with the gene expression data now available in the MET database.

Interestingly, specimens obtained from mouse testis and epididymis showed biological activity for NGF when tested in explants from sympathetic ganglia. The significance of these observations is not yet clear, although it has been postulated that epididymal NGF may play a role in sperm maturation (Ayer-LeLievre et al, 1988). This possibility is supported by the large number of signaling mechanisms in which NGF and other neurotrophins are involved; not only in neurons, but other cell types as well (Huang and Reichardt, 2003). Furthermore, a role of NGF in the development of mouse testis and epididymis has also been postulated on the basis of observed spatiotemporal patterns in expression of NGF and NGF receptors (Russo et al, 1999).

Platelet-derived growth factor (PDGF) and the PDGF receptor (PDGFR) are also present in the rat and mouse epididymis of both prenatal and postnatal animals (Basciani et al, 2004). The PDGF family comprises a series of 30-34-kd homodimers and heterodimers formed by a combination of 4 polypeptides, PDGF-A, -B, -C, and -D, which bind to two different receptors, $\alpha$ and ß, and exhibit mitogenic activity mainly on connective tissue cells (Heldin et al, 2002). In the adult rat, principal cells show intense immunostaining along the length of the epididymis for PDGF-A, PDGF-B, PDGFR $\alpha$, and PDGFR ß. Basciani et al (2004) have also shown that, beginning at approximately 25 days of age, the epididymis of PDGF-A knockout mice exhibit profound structural alterations consisting of a reduction in size and an aberrant epithelium. PDGF-B and PDGF receptor ß knockout mice die immediately after birth and show no epididymal alterations. These authors have postulated, on the basis of previous observations suggesting that PDGF may influence procathepsin L in the epididymis of the boar (Okamura et al, 1995), that PDGF may be involved in the regulation of the secretion of this and, perhaps, other proteins by the epithelium of the epididymis.

The transforming growth factor-ß (TGF-ß) family comprises three 25-kd isoforms of TGF-ß (ß1, ß2, and ß3). They are secreted by certain tumor and normal cells and are capable of transforming nontumoral cells in culture. They have been shown to influence various morphogenetic processes, such as growth, differentiation, wound healing, and apoptosis (Shi and Massagué, 2003). It is known that TGF-ß is present in the testis (Caussanel et al, 1997) and influences both Leydig (Morera et al, 1987) and Sertoli cells (Esposito et al, 1991). Much less is known, however, about the role of TGF-ß in the epididymis.

It has been recently demonstrated through immunohistochemistry that TGF-ß1 and TGF-ß receptor type II are both present in the epithelial cells of the caput and corpus regions of the epididymis of the

marmoset monkey (Bomgardner et al, 1999). Whereas TGF-ß1 was localized to apical cells, the TGF-ß receptor type II was restricted to principal cells; but in both cases, a higher proportion of stained cells was observed in the caput than in other regions. RT-PCR confirmed TGF-ß1 expression in the epididymis (Bomgardner et al, 1999). These observations prompted the hypothesis that TGF-ß may have a paracrine role in the epididymis, possibly in relation to the effects of dihydrotestosterone and estrogens. Similar studies in the rat epididymis have shown that TGF-ß1 localization is mostly interstitial rather than epithelial, but failed to show any regional distribution. TGF-ß3 localization, on the other hand, is epithelial and almost exclusively restricted to the corpus (Desai et al, 1998). Additional analysis by Northern blotting confirmed these results and showed that the expression of TGF-ß3 mRNA in the corpus is 6-fold higher than that in the caput and cauda. Studies involving castrated rats also support the notion of an interaction between androgens and TGF-ß (Desai and Kondaiah, 2000). In these animals, TGF-ß1 mRNA expression (as detected by Northern blotting) increases dramatically in the corpus, approximately 10-fold relative to noncastrated animals, and somewhat less (3-4-fold) in the caput and cauda. Most importantly, these changes are reversed by testoterone treatment. TGF-ß2, which is normally undetectable, is upregulated except in the cauda, and TGF-ß3 expression increases in the caput, but is much less in the corpus and does not occur in the cauda. In the mouse, according to the MET database, the gene encoding TGF-ß1 is the form most prominently expressed, especially in segment 1.

The presence of insulin-like growth factor (IGF) in the epididymis was originally reported by Hanson et al (1988). IGFs I (originally named somatomedin C) and II are small proteins (approximately 22 kd in size) structurally related to proinsulin that play important endocrine, paracrine, and autocrine roles in healthy, tumoral, and developing tissues (Grimberg and Cohen, 2000). These biological effects are regulated by IGF binding proteins (IGFBPs) that limit the bioavailability of IGFs and, in some cases, behave as IGF antagonists. A detailed immunochemistry study of the distribution of IGF I in the rat epididymis has shown that, at early postnatal ages (2-4 weeks), immunostaining is localized to epithelial cells (Leheup and Grignon, 1993). At later ages (4-8 weeks), immunostaining, particularly in the cauda, is less prominent in the epithelium, and more intense in myofibroblastic cells. Expression of the genes encoding IGF and IGFBPs is not greater than background levels in the adult mouse epididymis (MET database). Studies of the distribution of an IGFBP-rP1, however, show that this protein is present in the epithelium of the human epididymis (Degeorges et al, 2000). The significance of these results is unknown, but it is possible that in some species, IGF I may have a role in the epididymis.

It has recently been reported that erythropoietin (Epo) is produced by the mouse epididymis (Kobayashi et al, 2002). RT-PCR shows that the epididymis expresses Epo mRNA at relatively high levels, approximately 40% of the levels observed in the kidney. Secretion of Epo was confirmed by incubating epididymal tissue for 2-8 hours in the presence and the absence of the protein synthesis inhibitor cycloheximide. Levels of Epo in the culture medium, as revealed by ELISA, were greatly reduced by this drug. Interestingly, Epo production was independent of testosterone and estradiol. In situ hybridization shows that Epo is likely to be produced by interstitial cells. Epo is a 34-kd glycoprotein secreted by the fetal liver and the adult kidney that is essential for erithropoiesis because it stimulates bone marrow erythroid progenitors to proliferate and differentiate (Jelkmann, 1992). Although the kidney is the most important source of Epo during adulthood, other tissues, such as the brain and female reproductive organs, are also sources (Ebert and Bunn, 1999). Interestingly, the temporal pattern of Epo secretion by the epididymis resembles that of the kidney: there is an initial peak, followed by a phase of downregulation, which contrasts with the response of other organs that exhibit a more sustained secretory response (Kobayashi et al, 2002). Accordingly, it has been speculated that the epididymis may be a source of circulating Epo (Kobayashi et al, 2002). This notion has recently been supported by the demonstration of the presence of hypoxia-inducible factor-

1 in the rat epididymis (Palladino et al, 2004), which is a transcription factor that activates several genes involved in oxygen homeostasis, including *Epo*. The gene encoding Epo is not represented in the MET database, but Epo receptor is expressed in all 10 epididymal segments (not shown).

Finally, it has been suggested that hepatocyte growth factor (HGF) may be a mediator of the increased motility attained by sperm cells during epididymal transit. HGF is a potent angiogenic and tumor-promoting substance found in a variety of tissues (Jiang et al, 2005). Experiments in rats show that as sperm cells move from the caput to the cauda epididymis, they show a more widespread distribution of c-met, a receptor for HGF (Catizone et al, 2002). This has been correlated with previous observations involving mice, in which HGF was detected by immunohistochemistry in the mouse distal corpus and cauda, but not in the caput, and with the fact that HGF initiates motility of caput—but not cauda—epididymis sperm cells (Naz et al, 1994). Moreover, Catizone et al (1994) have shown that the motility of rat caput sperm cells is maintained in vitro by the presence of HGF. These investigators believe that HGF may be important in the acquisition of sperm cell motility in the epididymis because these cells are exposed to higher concentrations of HGF as they move to the cauda, and in addition, because they may be more sensitive to this substance, owing to the increase in c-met. The fact that sperm cells are immotile in situ in the cauda epididymis of most species studied suggest that this relationship is of a more complex nature (Turner and Reich, 1985; Turner et al, 2006).

## *Concluding Remarks*

The studies reviewed here have contributed to a body of information related to possible roles of growth factors in the epididymis. They are valuable in that they have revealed the presence and distribution of different growth factors in the epididymis of different species and have implicated potential biological roles for these substances. Unfortunately, for the most part, these studies have not gone far beyond that. Moreover, given the scatter of the data across different species and growth factors, and the fact that multiple studies have rarely focused on a single theme, it has not been possible to envision a unified theory accounting for the bulk of observations reported. A consistent data pattern has not emerged, and in many cases, it is not clear that observations in one species can be extrapolated to another. Obviously, the role of growth factors in the epididymis cannot be ascertained from localization studies alone. Rather, more mechanistic and functional studies, such as those performed by Ergün et al (1998) and Hinton and colleagues (Lan et al, 1998; Kirby et al, 2003), are required.

In this connection, an important aspect of the biology of growth factors remains mostly unexplored: what are the intracellular signaling cascades activated by growth factors in the healthy and the diseased epididymis? Although we know, for example, that the members of the *raf* and *myc* families are expressed in the mouse epididymis (Winer et al, 1993; Cornwall et al, 2001), much more information about these and other intracellular mediators is required to understand the biological role of growth factors on the epididymis.

Our current understanding of the physiology of the epididymis indicates that the epididymal tubule is regulated within segments (Turner et al, 2003; Johnston et al, 2005). Therefore, future investigations can be envisaged that study the role of growth factors within those separate segments. For instance, are there sharp differences in how different segments respond to paracrine or lumicrine factors? In the above mentioned cases involving *raf* and *myc*, it was demonstrated that A-*raf* and B-*myc* expression have segment-specific patterns and depend on androgens and testicular factors (Winer and Wolgemuth, 1995; Cornwall et al, 2001). It is also likely that the idiosyncratic nature of ERK activation may play a role in the biology of the epididymis. As noted elsewhere, ERK

activation is a complicated phenomenon requiring specific temporal and spatial arrangements of kinases and other molecules (Schaeffer and Weber, 1999 for review), and often cross talk with other signaling systems, such as $Ca^{2+}$-regulated cascades (Agell et al, 2002).

Although further investigations are needed to understand the role of growth factors in the epididymis, the work already completed has helped to delineate possible avenues for further research. On the one hand, a great deal of circumstantial evidence has been provided in support of the notion that growth factors play a role in epididymal physiology. These leads should be pursued, emphasizing the functional aspects of growth factors at the cellular and subcellular levels. On the other hand, the concept of epididymal segmentation provides a useful paradigm to further refine our understanding of this organ. As the mouse epididymal transcriptome substantiates epididymal segments, either alone or in concert with adjacent segments, form functional units exhibiting unique patterns of gene expression are likely to be differentially influenced by selected growth factors. The convergence of these two approaches may prove to be effective for further defining the biological effect of growth factors on the epididymis, and ultimately, for increasing our understanding of the ability of the epididymis to promote sperm maturation.

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