Human testicular peritubular cells: more than meets the eye

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Abstract

In healthy men, several layers of inconspicuously flat cells and extracellular matrix (ECM) proteins build the wall of the seminiferous tubules. The cells of this wall, peritubular cells, are not well characterized. They are smooth-muscle-like and contractile and transport immotile sperm, a function important for male fertility. However, their full functional importance, especially their potential contribution to the paracrine regulation of the male gonad, is unknown. In men with impaired spermatogenesis, the architecture of the tubular wall is frequently altered. Deposits of ECM and morphological changes of peritubular cells imply that functions of peritubular cells may be fundamentally altered. To be able to study human peritubular cells and their functions, a culture method was established. It is based on small biopsies of patients with obstructive azoospermia but normal spermatogenesis (human testicular peritubular cells, HTPCs) and non-obstructive azoospermia, impaired spermatogenesis, and testicular fibrosis (HTPCFs). Results obtained from cellular studies and parallel examinations of biopsies provide insights into the repertoire of the secretion products, contractile properties, and plasticity of human peritubular cells. They produce ECM components, including the proteoglycan decorin, which may influence paracrine signaling between testicular cells. They may contribute to the spermatogonial stem cell niche via secreted factors. They are regulated by mast cell and macrophage products, and in response produce factors that can fuel inflammatory changes. They possess a high degree of plasticity, which results in hypertrophy and loss of contractile abilities. The data collectively indicate important roles of inconspicuous testicular peritubular cells in human male fertility and infertility.

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Peritubular cells of the testis: of mice and men

The wall of the seminiferous tubules in the adult human testis is a rather small compartment within the adult male gonad, which has not received much attention (Maekawa *et al.* 1996). Striking species differences are obvious. In man, several layers of elongated, very thin, spindle-shaped, peritubular cells and extracellular matrix (ECM) proteins form this compartment. This is in contrast with the single cell layer and negligible ECM, which form the tubular wall in laboratory rodents. In either case, peritubular cells in the adult express markers for smooth-muscle-like cells, e.g. smooth muscle actin (Anthony & Skinner 1989, Tung & Fritz 1990, Albrecht *et al.* 2005). Differences in the cellular phenotypes between the inner and outer layers in man were suggested, according to results of immunohistochemical studies. The inner layers stained with desmin, supporting their smooth muscle phenotype, while the outer ones stained mainly with vimentin, implying a connective tissue phenotype (Davidoff *et al.* 1990). The smooth muscle characteristic, i.e. expression of smooth muscle actin, appears to be induced around puberty in primates mainly by androgens, which appear to play a crucial role

in the differentiation of peritubular cells. FSH has an additive role, implying that both Leydig cells and Sertoli cells may govern differentiation of peritubular cells. Once induced, smooth muscle actin expression appears to remain unchanged, even if the hormonal stimulation is removed (Schlatt *et al.* 1993).

The effects of androgens on peritubular cells may go beyond the induction of the structural contractility protein smooth muscle actin. This can be concluded from recent studies, in which the androgen receptor of murine peritubular cells was selectively knocked out (Welsh *et al.* 2009). As a consequence, the mice were azoospermic and infertile. Thus, peritubular cells are probably essential for the development of overall testicular function, spermatogenesis, and fertility, at least in rodents (see also Nurmio *et al.* (2012)).

How peritubular cells are involved in the orchestration of testicular functions in the adult remains to be fully shown. The anatomical 'strategic' position of peritubular cells within the testis, namely at the interface of the interstitial, i.e. Leydig cell compartment, and the Sertoli cell/germ cell, i.e. tubular compartment, intuitively suggests multiple paracrine roles of these cells within

the testis. Indeed, several studies revealed important paracrine influences exerted by peritubular cells on somatic cells. In mice, in which the peritubular cell androgen receptor was knocked out, Leydig cell markers (e.g. steroidogenic factor 1, IGF and IGF3) were significantly reduced in a certain population of Leydig cells (Welsh *et al.* 2012). Thus, Leydig cells are among the somatic cells that are under the control of factors derived from peritubular cells.

That Sertoli cell function was also impaired in the knockout mice mentioned does not come as a surprise. Earlier reports postulated paracrine interactions and the factor involved was termed 'peritubular factor that modulates Sertoli cell function' (Skinner & Fritz 1985). This probably represents a cocktail of different factors, which have not been further characterized (Verhoeven *et al.* 2000).

Sertoli cells are of crucial importance for all male germ cells and are thought to provide the structural and functional environment for the spermatogonial stem cell (SSC). An influence of peritubular cells on Sertoli cells may therefore have consequences also for the SSC. Peritubular cells and SSC are separated only by a basal lamina and hence peritubular cells may also directly contribute to the SSC niche, e.g. via secreted factors (Oatley & Brinster 2012).

An important and accepted role of peritubular cells is the active transport of immotile sperm (Hargrove *et al.* 1977). A few years ago, it was even speculated that interference with the contractile abilities of seminiferous tubules, i.e. cycles of contraction, and relaxation of peritubular cells, may be a strategy for human male contraception (Romano *et al.* 2005). In previous mouse studies (Zhang *et al.* 2006, Welsh *et al.* 2009), the lack of androgen receptors in peritubular cells most probably prevented the functional maturation of these cells, which normally occurs at puberty. It therefore appears possible that the consequent lack of contractility of seminiferous tubules may also have contributed to the observed infertility. Unfortunately, this was not tested.

Factors involved in the regulation of contraction and relaxation of peritubular cells appear numerous. They were studied both in rodent isolated peritubular cells and in human isolated whole seminiferous tubules. Local factors, e.g. endothelin 1 (Romano *et al.* 2007, Losinno *et al.* 2012), platelet-derived growth factor (PDGF; Romano *et al.* 2006), prostaglandins, and neurotransmitters (Miyake *et al.* 1986, Tripiciano *et al.* 1998), may play a role, as do hormones, including sex steroids (Yamamoto *et al.* 1989) and the pituitary hormone vasopressin. In rat peritubular cells, receptors for vasopressin (V1a), in contrast to endothelin receptors, appear to be under developmental control. They are present in the adult rat, but absent before puberty (Howl *et al.* 1995).

In this context, it is important to bring to mind that the tubular wall compartment in subfertile or infertile men

with impaired spermatogenesis is typically remodeled. It is textbook knowledge that testicular tubular fibrosis, best evidenced by deposits of ECM, is considered a hallmark of male infertility. Fibrosis and morphological changes of peritubular cells, including hypertrophy, reported by several studies (Haider *et al.* 1999, Schell *et al.* 2010, Welter *et al.* 2013), may imply that specific functions of peritubular cells, possibly including paracrine and contractile abilities, are altered (Figs 1 and 2). If so, the following questions arise: what is/are the reason(s) for the changes, what may initiate and drive the changes, and do they indeed affect testicular function?

The reasons for the changes may be related to intrinsic plastic properties of smooth muscle cells. Typical smooth muscle cells, e.g. from the vasculature, are well known for their ability to undergo dramatic phenotypic changes due to a plethora of influences, including inflammatory factors (Alexander & Owens 2012, Welter *et al.* 2013). As testicular peritubular cells share characteristics of smooth muscle cells, the changes in the tubular wall occurring in the testis of infertile men could simply indicate phenotype switches. In support of this, adult rodent peritubular cells in culture were reported to undergo striking changes, e.g. in response to endothelin and PDGF, which cause hypertrophy (Romano *et al.* 2006, 2007). Thus, testicular peritubular cells are able to respond to stimulation by a phenotypic adaptation.

What may trigger the phenotype changes in the human testis? Recently, another alteration in the wall of human seminiferous tubules in infertility patients became clear, namely the presence of abundant immune cells, i.e. macrophages and mast cells (Fig. 1; Hashimoto *et al.* 1988, Nagai *et al.* 1992, Apa *et al.* 2002, Hussein *et al.* 2005, Roaiah *et al.* 2007). Thus, mast cell-specific genes are strikingly and robustly increased in testes samples from infertile men (Spiess *et al.* 2007). Immunohistochemistry combined with morphometric studies and electron microscopy revealed that activated mast cells specifically accumulate in the wall of those seminiferous tubules, which display impaired spermatogenesis (Meineke *et al.* 2000, Frungieri *et al.* 2002a, Welter *et al.* 2011). Tryptase is a prototype mast cell marker and the number of tryptase-immunoreactive mast cells was positively correlated with the thickness (i.e. the extend of fibrosis) of the walls of seminiferous tubules (Meineke *et al.* 2000). In addition, increased numbers of CD68-positive macrophages were found (Frungieri *et al.* 2002b). Hence, the cellular and extracellular composition (i.e. fibrosis) and consequently the local microenvironment of afflicted tubular walls are altered in states of human male infertility. Although not examined, interactions between immune cells and peritubular cells could result in a phenotype switch of peritubular cells, in fibrosis of the tubular wall (Levi-Schaffer & Piliponsky 2003, Duchesne *et al.* 2011) and other unexplored changes.

Figure 1 Testicular morphology and composition of the tubular wall in the adult human testis in health (normal spermatogenesis; A and C) and disease (infertility B and D). (A) The tubular wall (arrows) is normally an inconspicuous compartment, in which only few nuclei of peritubular cells are visible in hematoxylin and eosin (H&E)-stained sections. Note the close proximity of the tubular wall to the tubular compartment (TC) containing Sertoli cells and germ cells and to the interstitial compartment (ISC) with Leydig cells. Bar: $\sim 30 \mu\text{m}$. (B) In samples from infertility patients, the tubular wall is often enlarged, deposits of ECM are seen, and both elongated and round peritubular cell nuclei (arrows) are seen in the thickened wall. H&E stain. Bar $\sim 30 \mu\text{m}$. (C and D) Immunohistochemical identification of mast cells containing tryptase, in a normal testis (C) and in a testicular biopsy of an infertility patient (D). Note that in the section of the normal testis, only one mast cell is present in the interstitial compartment (C). In contrast, in the section from the infertility patient, several mast cells are seen and accumulate in the wall of seminiferous tubules (arrows in D). Bar $\sim 60 \mu\text{m}$.

All these changes may be of importance for spermatogenesis and human male fertility. But how can human peritubular cells and the tubular wall be studied? As mentioned, the rodent tubular wall has a simple architecture, lacks appreciable ECM and immune cells. Hence, peritubular cells in rodents may differ functionally from human peritubular cells. This point can, however, not be fully judged, because despite several studies on rodent peritubular cells, only very few papers dealing with human testicular peritubular cells (HTPCs) have appeared. As a further caveat, these few studies examined samples from boys with cryptorchidism or androgen insensitivity syndrome (Chemes *et al.* 1985, 1992, Cigorraga *et al.* 1994) and therefore would not reflect the situation of the adult human testis. A recent case report indicates that peritubular cells of the human testis may be able to form tumors (Du *et al.* 2012). This benign testicular tumor type is very rare and only six

additional cases have been reported (see Du *et al.* (2012)). While peritubular tumor cells might turn out to be an interesting experimental tool in the future, they are, to my knowledge, not yet available for cellular studies.

Hence, to be able to explore HTPCs in health and disease, i.e. infertility, one has to develop adequate human cellular models. In an attempt to do so, our laboratory established a method to culture and study HTPCs. It is based on very small testicular tissue samples from adult patients with obstructive azoospermia but normal spermatogenesis (HTPCs) and non-obstructive azoospermia, impaired spermatogenesis, and testicular fibrosis (HTPCFs) (Albrecht *et al.* 2006, Schell *et al.* 2008, 2010, Spinnler *et al.* 2010, Adam *et al.* 2011, 2012).

Insights obtained from any cellular model must be viewed with caution in general, and our data in particular, because similar studies on human peritubular cells coming from other laboratories are, to my

Figure 2 Electron microscopy of human peritubular cells in the wall of seminiferous tubules. (Top) In the healthy adult, several layers of thin, peritubular cells (arrows at nuclei) and ECM form the wall of seminiferous tubules. Bar: $\sim 3 \mu\text{m}$. (Bottom) The morphology of the tubular wall of an infertility patient reveals cellular hypertrophy (arrow at nucleus) and increased ECM (asterisk). Bar: $\sim 4 \mu\text{m}$. These micrographs are taken with permission from Fig. 2 of our previous paper (Schell *et al.* 2010). Copyright 2010, reproduced with permission from The Endocrine Society.

knowledge, not yet available. Are HTPCs and HTPCFs indeed good models? First of all, one must bear in mind that HTPCs, derived from men with obstruction of the vas deferens, despite normal spermatogenesis and testicular morphology, may not necessarily be completely normal cells. Yet other samples are not available. Secondly, various underlying and often unknown causes for infertility in case of HTPCFs may complicate the interpretation of the results. Thirdly, due to the explant culture approach, the cells examined may represent only a very viable subpopulation of the cells, which build the tubular wall.

Bearing all this in mind, we have complemented all cellular studies by parallel examinations of human biopsy samples using immunohistochemistry and/or laser microdissection, followed by RT-PCR analyses. Thus, by monitoring the *in vivo* situation, we can hopefully detect mere cellular phenomena. When done in combination, we strongly feel that the study of the cellular models provides a valuable experimental avenue to a better understanding of adult human

peritubular cells. In this short review, key results are summarized. The focus is on unknown characteristics of peritubular cells and the role of immune cells.

Recent insights into the nature of human peritubular cells

The basic phenotype of HTPCs

The basic make-up of HTPCs was summarized previously (Albrecht *et al.* 2006, Albrecht 2009) and together with new insights shall be described. A short summary is provided in Table 1. Gene expression studies, confirmed by other methods, including western blotting, immunohistochemistry with biopsies, immunocytochemistry, RT-PCR, and ELISA techniques, revealed aspects of the basic make-up of HTPCs and HTPCFs. Their cellular phenotype is characterized by smooth muscle proteins, including smooth muscle actin, myosin heavy chain 11 (MYH11), calponin, cGMP kinase 1 (cgKI), and other smooth muscle markers (Schell *et al.* 2010, Welter *et al.* 2013). Examples of ECM proteins produced by HTPCs are several types of collagens, fibronectin, laminin, and secreted protein acidic and rich in cysteine (SPARC (osteonectin)) but also include the proteoglycan decorin (Albrecht *et al.* 2006, Adam *et al.* 2011, 2012). They produce signaling factors, including interleukin 6 (IL6), monocyte chemotactic protein 1 (MCP1), nerve growth factor (NGF), and its precursor proNGF, as well as glial cell line-derived neurotrophic factor (GDNF). Cyclooxygenase-2 (COX2) was detected upon $\text{TNF}\alpha$ treatment and prostaglandins, especially PGD2, and metabolites may also be among the produced factors (Schell *et al.* 2008, Spinnler *et al.* 2010). The cells express several antioxidant proteins, namely catalase, parkin/DJ-1, peroxiredoxin 1, superoxide dismutases (SOD) 1 and 2, glutathione-S-transferase, and heme oxygenase (HMOX1) (Kampfer *et al.* 2012). Finally, they possess receptors (R) for growth factors (GFs) including platelet-derived GFR and epidermal GFR (EGFR; see below), receptors for histamine (H1), $\text{TNF}\alpha$ (TNFR1/2), and tryptase (PAR2) (Albrecht *et al.* 2006, Schell *et al.* 2008, Adam *et al.* 2011, 2012). They also express androgen receptors (unpublished data) and peroxisome proliferator-activated receptor- γ (Schell *et al.* 2010) and the intercellular adhesion molecule, ICAM-1.

Interestingly, to date, only two known and distinct differences between HTPCs and HTPCFs have become detectable, namely that HTPCFs produce significantly more decorin and catalase compared with HTPCs. This most probably reflects an altered *in vivo* situation, which persists *in vivo* and provides a unique, albeit small window into the human testis in health and disease, i.e. infertility (Adam *et al.* 2011, Kampfer *et al.* 2012). We anticipate that upon completion of ongoing proteomic studies, we will have information about the

Table 1 Secreted products, cellular components, and receptors of human testicular peritubular cells.

Name	Description	Regulation
SMA	Structural protein	15dPGJ2/ROS
MYH11	Structural smooth muscle-specific protein, contraction	15dPGJ2/ROS
Calponin	Structural smooth muscle-specific protein, contraction	15dPGJ2/ROS
cGKI	Kinase involved in smooth muscle relaxation	?
DCN	Secreted product, interacts with collagens, binds growth factors (e.g. PDGF), ligand for growth factor receptors (e.g. EGFR)	Increased by tryptase, TNF α (only in HTPCFs)
MCP1	Secreted chemoattractant factor for monocytes	Increased by TNF α
IL6	Secreted inflammatory cytokine	Increased by TNF α
NGF/proNGF	Secreted factor with roles in innervation, mast cell survival, meiosis	Increased by TNF α ; proNGF/NGF affected by tryptase
GDNF	Secreted factor important for spermatogonial stem cells	Constitutive release
Catalase	Antioxidant action	ROS?
Peroxiredoxin 1	Antioxidant action	?
SOD 1/2	Antioxidant action	?
Park7/DJ-1	Antioxidant action	?
EGFR (family)	Receptor family	Proliferation, intracellular Ca ²⁺ ; signaling inhibited by DCN
PDGFR α / β (family)	Receptor family	Proliferation, intracellular Ca ²⁺ ; DCN blocks signaling by binding of ligand
PAR2	Receptor	Increases intracellular Ca ²⁺ ; activation by mast cell tryptase
H1	Receptor	Increases intracellular Ca ²⁺
TNFR1/2	Receptor	MAPK, regulates MCP1, IL6
PPAR γ	Receptor	Unknown
AR	Receptor	Unknown

AR, androgen receptor; cGKI, cGMP-dependent protein kinase 1; DCN, decorin; EGFR, epidermal growth factor receptor; GDNF, glial-cell-line-derived neurotrophic factor; IL6, interleukin 6; MCP1, macrophage chemoattractant protein-1; MYH11, myosin heavy chain 11; Park/DJ1, parkin/DJ-1; PAR2, protease-activated receptor 2; PDGFR, platelet-derived growth factor receptor; PPAR γ , peroxisome proliferator-activated receptor-gamma; Pro/NGF, pro/nerve growth factor; SMA, smooth muscle actin; SOD1/2, superoxide dismutases 1/2; TNFR1/2, tumor necrosis factor receptor 1/2.

complete protein repertoire of these cells and their secretome.

It is of importance to note that the phenotypic features of peritubular cells (e.g. smooth muscle proteins) and the secreted products (e.g. NGF) are not to be regarded as fixed. As will be dealt with in the following sections, peritubular cells respond to immune cell derived factors, resulting in striking changes in their phenotype and their secreted factors (Fig. 3).

HTPCs secrete the proteoglycan decorin, which acts as a paracrine factor and immune cell-derived factors stimulate decorin production

Decorin was described in the human testis and specifically in the tubular wall years ago (Ungefroren *et al.* 1995). This proteoglycan interacts with collagens, 'decorates' collagen fibrils, and thus has defined structural roles in the ECM. Decorin is often increased in human organ fibrosis. In line with this, we also found stronger and more widespread expression in testes of infertility patients, which also showed testicular tubular fibrosis (Adam *et al.* 2011, 2012). Decorin has, however, important additional functions, including regulation of inflammation (Merline *et al.* 2011). Furthermore, decorin is an ECM factor, which is involved in storing GFs, and due to this ability, it can lower the levels of bioactive factors in tissues. In addition, it can directly

bind to certain GF receptors. This is well known for the EGFR, which is expressed by peritubular cells and other cells in the human testis. In recent studies, we explored whether decorin may exert such actions also in HTPCs (Adam *et al.* 2011, 2012). Exogenous human recombinant decorin increased, like EGF, intracellular Ca²⁺ levels within seconds and phosphorylated EGFRs of HTPC/Fs within minutes. Subsequently, decorin blocked EGFR signaling, as evidenced by its action to abolish the mitogenic actions of EGF in HTPC/Fs. It also blocked the mitogenic actions of PDGF, yet the mode of action involves binding of the ligand rather than direct actions at the receptor level.

Are the results obtained in a cell culture system of relevance to the human testis? In the *in vivo* situation, immunoreactive decorin is found in the interstitial ECM and especially in the ECM of the fibrotically remodeled tubular wall (Adam *et al.* 2011, 2012). EGFRs are found in peritubular and Leydig cells, and receptors for PDGF are expressed by multiple testicular cells types (Basciani *et al.* 2010). Thus, in infertile men's testes, substantially higher levels of DCN are found and therefore interference with testicular paracrine signaling must be assumed. This may directly and indirectly contribute to impaired spermatogenesis (Adam *et al.* 2011, 2012).

It is also noteworthy that the levels of testicular decorin in a non-human primate, the rhesus monkey, are inversely related to the state of fertility. High levels of decorin are

Figure 3 Summary view of factors regulating proliferation, secretion, contraction, and phenotypic plasticity of cultured human peritubular cells. Based on experimental results, EGF and PDGF can stimulate proliferation, actions blocked by decorin. Serum factors induce contraction of HTPCs. Immune cell-derived factors, namely tryptase and/or TNF α , induce secretion of several proteins and signaling factors. A PG metabolite (15dPGJ2), via ROS, causes cellular hypertrophy and loss of contractility, which can be blocked by an antioxidant (NAC) and is reversible upon removal of the metabolite. GDNF is secreted in a constitutive fashion.

found in immature animals, i.e. the physiological state of 'infertility' and they decrease when sexual maturity approaches. Could decorin be a local regulator of paracrine actions in the testis? Unfortunately, this possibility cannot be readily examined in rodents, because we found that both rats and mice express only very small amounts of decorin in their testes. This may also explain why mutant mice, in which decorin was knocked out, do not have an obvious testicular phenotype (Adam *et al.* 2011, 2012).

Higher levels of decorin in the ECM of tubular walls of infertile men may be brought about by increased production and/or reduced turnover. The enzyme tryptase is a prototype mast cell product, which acts on target cells via its receptor (PAR2). Macrophages and mast cells, as we found (Schell *et al.* 2008), are also a source of TNF α in the human testis. Functional PAR2 and TNFR1/2 are expressed by HTPCs/HTPCFs. Their ligands, tryptase and TNF α , stimulated the production and secretion of decorin by HTPCFs, which are derived from men with infertility and testicular fibrosis (Adam *et al.* 2011, 2012). Provided that higher testicular levels of decorin are related to impaired paracrine signaling and impaired spermatogenesis, immune cells can contribute in this way to male infertility (Fig. 3). Circumstantial evidence for such a link is derived from

studies in a mutant mouse (aromatase overexpressing transgenic mouse) (Li *et al.* 2006). These animals become infertile with age. The testicular changes observed after about 3 months of age onward include impaired spermatogenesis, testicular fibroses, abundant macrophages, very high testicular TNF α levels, and very high decorin levels. Thus, male infertility in this mouse is inversely related to high levels of testicular decorin (Adam *et al.* 2011, 2012). Of course, a causal link remains to be proven.

HTPCs secrete the neurotrophic factors (pro)NGF and GDNF

The physiological importance of the prototype neurotrophic factor NGF in the human testis is not known (Seidl *et al.* 1996). Possible roles have been suggested, including involvement in meiosis in rodents (Perrard *et al.* 2007, 2009). NGF levels may furthermore be related to the sympathetic innervation of the human testis, which may increase in male infertility (Mayerhofer *et al.* 1999). NGF may also act on mast cells, e.g. by preventing their apoptosis (Welker *et al.* 1998, Kanbe *et al.* 2000, Meineke *et al.* 2000, Dutta *et al.* 2011).

In contrast, another neurotrophic factor, GDNF, is an established factor and is crucial for the renewal of SSCs in a wide range of species (Oatley & Brinster 2012). Importantly, the neurotrophic factors GDNF and NGF are produced by HTPCs/HTPCFs (Schell *et al.* 2008, Spinnler *et al.* 2010, 2011). NGF is normally secreted in low amounts by HTPCs, but TNF α strongly stimulates both expression and secretion levels, as measured by RT-PCR and ELISA in cell culture supernatants respectively. Hence, testicular macrophages and mast cells, which contain TNF α and are present in the tubular wall, could regulate NGF production by peritubular cells via TNF α . Consequently, diverse downstream events (e.g. innervation, number of mast cells, and spermatogenesis) (Schell *et al.* 2008) may be altered.

In cellular experiments, we found that the mast cell product tryptase also increased the measurable amounts of NGF in cell culture supernatants. The reason for this increase is, however, distinct from the one described above. When we tested a peptide-ligand for PAR2, which lacks the enzymatic activity of tryptase, no stimulatory effect was observed. This puzzling result led to the discovery that the enzyme tryptase cleaves the precursor of NGF, proNGF (Spinnler *et al.* 2011), which is therefore also secreted by peritubular cells. Tryptase is a specific mast cell product, which does not diffuse far from the site of its secretion. Therefore, mast cells are probably able to modulate the proNGF/NGF levels in their vicinity and thus tubular wall mast cells may influence local NGF- and proNGF-dependent events in the human tubular wall (Fig. 3). Unfortunately, there is no information at all about a role of proNGF in the testis, but its actions are linked to cell death in many organs

including for instance the human heart, in which it causes microvasculature damage (Siao *et al.* 2012).

GDNF was found to be a secretory product of both HTPCs and HTPCs, which produce this factor in a constitutive fashion and in similar concentrations, as shown by ELISA measurements (Spinnler *et al.* 2010). Neither tryptase nor TNF α affected the levels of secreted GDNF. Additional studies (namely RT-PCR of laser-microdissected areas of human biopsy material) were performed because suitable antibodies for immunohistochemistry were not available. Results obtained imply that GDNF is probably produced by human peritubular cells and by Sertoli cells of the human testis *in vivo*. The latter cells are accepted testicular producers of this factor in many species. SSCs express a receptor for GDNF, GFR α -1, which is readily found in some human basal spermatogonia, which therefore may represent SSCs. Given the anatomical proximity of peritubular cells and SSCs, separated by a basal lamina, paracrine interactions may readily occur. These results therefore have led to the hypothesis that the cells of the tubular wall contribute directly to the SSC niche in the human testis. This idea is currently being explored.

HTPCs, immune factors and reactive oxygen species: phenotypic modulation of the contractile state

In HTPCs, TNF α stimulated the normally low production of pro-inflammatory IL6 and of MCP1 (Schell *et al.* 2008). MCP1 may be involved in the observed increase in testicular macrophages in infertility patients. Together, with elevated mast cell numbers, more macrophages provide morphological evidence for an inflammatory testicular milieu, which is reported in many cases of male infertility (Schuppe *et al.* 2008).

A number of soluble factors may be actors in such a microenvironment. They probably include prostaglandins, which are produced by Leydig cells and macrophages in human testes with massively impaired spermatogenesis (Frungieri *et al.* 2002a, Matzkin *et al.* 2010). They are also most probably derived from mast cells, albeit only in certain states of partial degenerating of seminiferous tubules. In these 'mixed-atrophy' samples, tryptase-positive mast cells express the prostaglandin-synthesizing enzyme cyclooxygenase 2 (COX2) (Welter *et al.* 2011). The actions of prostaglandins in the human testis remain to be explored (for review, see Schell *et al.* (2007, 2010) and Kampfer *et al.* (2012)).

Interestingly, an end-product of non-enzymatic prostaglandin metabolism, 15-deoxy-prostaglandin J2 (15dPGJ2), had a strong influence on cultured HTPCs and HTPCFs (Kampfer *et al.* 2012). The metabolite induced the generation of reactive oxygen species (ROS), which consequently resulted in a striking phenotypic switch of HTPCs (Schell *et al.* 2010, Welter *et al.* 2013). The cells became hypertrophic and showed a diminished expression of smooth muscle cell markers

(e.g. smooth muscle actin, MYH11, or calponin). This change was associated with a reduced ability to contract upon addition of serum factors, as examined in collagen gel contraction assays. The antioxidant *N*-acetyl-cysteine (NAC) prevented hypertrophy and loss of smooth muscle markers and of contractility. Furthermore, upon removal of 15dPGJ2, cells spontaneously reverted to the normal phenotype, indicative of a high intrinsic degree of cellular plasticity.

Is this of relevance for the human testis? Similar phenotypic changes of peritubular cells, namely hypertrophy and reduced smooth muscle marker expression, evolve as common alterations, observed in the testes of infertile men (Schell *et al.* 2010, Welter *et al.* 2013). In human testicular samples from infertility patients, 15dPGJ2 can be detected (Kampfer *et al.* 2012). Circumstantial evidence for increased ROS levels in the tubular wall of infertility patients recently evolved from the comparison between HTPCs and HTPCFs. The latter express higher levels of the H₂O₂-metabolizing enzyme catalase and when challenged with H₂O₂ respond more effectively. It is thus possible that COX2 expression, prostaglandin formation, and metabolism, which induce the generation of ROS, are interrelated events, forcing smooth muscle-like peritubular cells to adapt and change their phenotype (Fig. 3). If contractility of seminiferous tubules is indeed crucial for sperm transport and fertility, the loss of contractility is an overlooked factor contributing to male infertility.

Figure 4 Summary of hypothetical roles of peritubular cells in the human testis. Human peritubular cell may be both sources of and targets for factors involved in the regulation of testicular cells. Leydig cells, Sertoli cells, SSC, and neighboring peritubular cells, as well as immune cells, e.g. mast cells and macrophages, may communicate via secreted proteins or other signaling factors.

Where do we stand and where should be go?

The position of the tubular wall within the testis implies that its cells should be able to interact with almost all cell types of the testis, provided that they secrete signaling molecules and possess relevant receptors (Fig. 4). In the case of the human testis, the cellular models of HTPCs and HTCfs and the exploitation of biopsy material are combined approaches, which allow us to decipher such factors and receptors and provide hints of specific roles of peritubular cells in the adult human testis in health and disease. An intriguing result is the unexpected high degree of cellular plasticity, which includes contractile and secretory properties and the insight that peritubular cells are targets of immune-cell-derived factors. The insights may be of translational relevance with regard to treatment of male subfertile or even infertility and with regard to male contraception.

First, if the phenotype of peritubular cells and their functions is subject to regulation by immune cells, is there a safe and effective way to intercept signals from testicular immune cells in subfertility/infertility patients? One approach, the pharmacological inhibition of testicular mast cells, was indeed explored in the past in a limited number of subfertility patients. The reported results were, in general, promising and describe amelioration of subfertility (Matsuki *et al.* 2000, Azadi *et al.* 2011, Haidl *et al.* 2011). Large, controlled, and randomized studies are, however, urgently required in the future to better address this point and to extend it beyond mast cells.

Secondly, is there a safe way to reversibly modulate the contractile and/or secretory phenotype of peritubular cells and would that approach work for human male contraception? Before these points can be clarified, however, much more is to be learned about HTPCs, which appear to be the least explored cells of the human testis.

There are further issues of considerable scientific and clinical interest involving peritubular cells, which were not mentioned in this short paper. They include, for example, the new question whether peritubular cells may have a role in the formation of the testis, as was suggested by the results of a recent rodent study (Nurmio *et al.* 2012). Answers to an old question, whether the peritubular cell population may contain Leydig cell progenitor or even stem cells, as implied by several studies in rodents, may be provided. Finally, the study of human peritubular cells may help to clarify their potential contribution to germ cell tumor development and progression, which is an emerging topic of clinical relevance (Diez-Torre *et al.* 2011).

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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