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Histological and Functional Organization in Human Testicle: Expression of Receptors c-kit and Androgens

Organización Histológica y Funcional en el Testículo Humano: Expresión de los Receptores c-kit y Andrógenos

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SUMMARY: The objective of this work was identify the presence of interstitial cells of Cajal, muscle cells, nerves and androgen receptor positive cells in adult human testicle, using immunohistochemical detection for c-kit/CD-117, actin smooth muscle specific (ASMS), neurofilament (N) and androgen receptor (AR), respectively. The samples were obtained from patients (n= 10) with diagnosis of prostate cancer, with surgery of orchiectomy. Subsequently were processed by histology and for immunohistochemistry using specific antibodies. It showed the presence of cells c-kit/CD-117, with diverse degrees of positivity, distributed mainly in the interstitial peritubular area of the human testicle. The peritubular myoides cells were positive to the presence of the actin smooth muscle and androgen receptor. The neurofilaments elements (+) only were observed in the vascular tunic. The specific immunohistochemistry describe the presence of the interstitial cells of Cajal in human testicular interstitium, opening a new perspective for the functional interpretation of the testicular cellularity and tubular motility. Possibly associated functionally to peribubulars cells of smooth muscle to regulate the mobility of the seminiferous tubules, whose integration and function would be androgen dependent. The cells that express the c-kit receptor, were found exclusively in the interstitial compartment. This cellular type in addition of the muscular cells of peritubules and the absence of nervous fibers to the interior of the testicle, could be responsible for the regulation of tubular mobility, as it happens in the gastrointestinal apparatus.

KEY WORDS: Human reproduction; Testicle; Interstitial Cajal cells; Tubular motility; AR.

INTRODUCTION

During the embryonic and fetal development of the testicle, a series of changes occurs in both cellular distribution and histological organization that lasts until it reaches sexual maturity. This developmental process ensures the optimal organization to produce masculine gametes and male hormones (Rodríguez *et al*., 2004).

Somatic cells of the testicle are Leydig cells, peritubular myoids cells and Sertoli cells. In these cells the presence of androgen receptors (AR) has been demonstrated, with variable immunohistochemical positivity according to the age and state of the cycle of the spermatogenesis.

The androgens mediate a wide range of physiological responses and are especially important in male sexual maturation, the maintenance of spermatogenesis, and male gonadotropin regulation (Carreau *et al*., 2007).

The effects of androgens are mediated through the androgen receptor (AR), a 110-kDa ligand-inducible nuclear receptor that regulates the expression of target genes through binding to an androgen response element. Mutations of the AR may result in male infertility or complete or partial androgen insensitivity (Brockschmidt *et al.,* 2007).

In the human testis, AR immunoexpression was observed in Sertoli cells, peritubular myoid cells, Leydig cells, and periarteriolar cells, but not in germinal cells. There

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is no correlation with the intensity of AR immunoexpression in either Sertoli cells or peritubular myoid cells in regard to spermatogenic cells. Perhaps an inappropriate expression of the AR is a cause or a consequence of idiopathic infertility in the human patients (Zhang *et al*., 2006; Loukil *et al.*, 2005).

The membrane receptor c-kit, a tyrosine quinase protein type III, directly regulates the proliferation and apoptosis of stem cells and it is involved in cell migration. In the embryofetal and immature testicle, the receptor would be expressed by the somatic cells (Sertoli and Leydig) and germinal cells (spermatogonia types A, Intermediate and B). Some authors even describe an incomplete c-kit protein in some meiotics stages (Ullrich & Schlessinger, 1990). Nevertheless, in the adult man there would take place a reduction in the number and type of cells that express the protein c-kit (of unknown function), which probably is an important factor in the regulation of the endocrine and gametogenic functions of the adult testicle. Therefore, the expression of the c-kit receptors may perhaps constitute one of the regulating factors of the spermatogenesis, by means of modulating proliferation and apoptosis in the seminiferous epithelium, as well as regulation of tubular motility (Rothschild *et al.*, 2003).

In the peritubular compartment, the myoid cells are androgen dependent, surround the seminiferous tubules and secrete basal lamina components (Jeanes *et al.,* 2005). These myoid peritubular cells with contractile properties could be regulating the motility of the seminiferous tubules.

Therefore the contractility of the seminiferous tubules and the displacement of the intratubular fluid could be regulated through testosterone specific receptor, and by the activity of the positive c-kit cells.

In the present work, in the interstitium of the adult human testicle we describe positive c-kit cells associated to the peritubular contractile cells, probably interrelated and regulated by testosterone.

MATERIAL AND METHOD

Testicular tissue was obtained from prostate cancer patients (n= 10, between 60 to 65 years old), under procedures of uni or bilateral testicular surgery (orchiectomy) corresponding to an androgen ablation therapy. The patients were asked to authorize the use of the tissues, after explaining the aims of the work. The Bioethical Committee, for handling human tissues, according to the Clinical Hospital José Joaquín Aguirre, of the University of Chile, authorized this study.

The samples of testicular tissue were fixed in 40 g/L buffered formaldehyde pH 7.4 in PBS (phosphate buffer saline). All staining procedures for light microscopy were performed on 4 mm paraffin-embedded sections and stained with hematoxylin-eosin (or Papanicolaou stain).

For detection of c-kit a rabbit antihuman antibody and horseradish peroxidase enzyme-labeled polymer conjugated to polyclonal rabbit secondary antibodies (LSAB-2; DAKO Corp.) was utilized. Similar protocols were performed with antibodies against Actin smooth muscle specific and neurofilaments (NeoMakers ref RB 9010-R7) and the androgen receptor (LabVision CO. Ref RB 9030 - PO), revealed with UltraVision Plus Detection System, Anti polivalente, HRP. All slides were incubated with the primary antibody for 10 minutes at RT (room temperature), then incubated with the secondary antibody, a biotinylated goat anti-rabbit for another 10 minutes at RT, and finally with Peroxidase-Streptavidin conjugated and with AEC (Aminoethylcarbazole) or DAB (Diaminobenzydine) as chromogen (DAB) to develop the color reaction. Endogenous peroxidase was inactivated by 3 % H_2O_2 for 5 minutes (30 % perhydrol p.a., Merck). Negative controls were performed by either blocking with appropriate non-immune serum or by omitting the primary antibody from the protocol.

The results were registered in digitalized images (digital camera Nikon® Coolpix 4500, 4 Megapixeles of resolution), and the quantification was made in a Nikon Microscope Eclipse. For the c-kit cells, AR, and smooth muscle specific actin, the number of immunopositives cells by seminiferous tubule was registered, considering a total of 1200 tubules.

RESULTS

Testicular histology usually considers an organization in compartments: tubular, peritubular and intertubular or interstitial. The cellularity in each one of them is different, and the functions are also different, with an intratesticular paracrine regulation, which assures the functional interdependence between the compartments.

In Tables I and Figs. 1 and 2, the quantitative results of the technique of immunohistochemistry of the testicular cells that express the protein c-kit, the androgen receptor and specific actin of smooth muscular are shown. In Table I, the quantification of the c-kit positive cells is observed, in the adult human testicle, assumed to be pacemarker cells, though scarce in number. They are found in association to structures that present contractile properties such as arterioles and, in greater amount, around the seminiferous tubules.

Table I. Quantification and distribution c-kit + cells in the testis, immunocytochemistry reaction by area (40x), with registration the position near seminiferous tubules or vascular area.

	Cells	**Distribution (%)**	
	Cell/ 0.25mm^2	Peritubular	Perivascular
c-kit (+) cells	0.4 ± 0.002	82	18

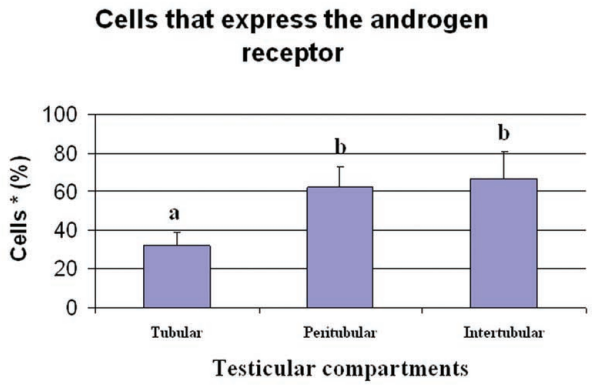

Fig. 1. Quantification and distribution of the cells of the compartments of the adult human testicle that express the androgen receptor (a,b: p≤ 0.05).

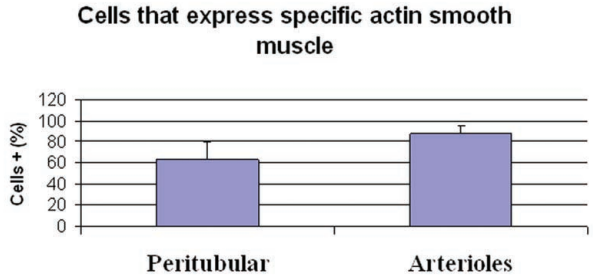

Fig. 2. Cells of human adult testicle that express specific actin of smooth muscle. Seminiferous tubules and vascular system.

In Fig. 1, in the different compartments of the adult human testicle there are different cellular populations that express the androgen receptor protein. Also the contractile peritubular cells express in a high percentage the androgen receptor. In Fig. 2, in the peritubule the cells express actin, specific protein of the smooth muscle, which allows to confirm that these cells are of muscular character and that they could be participating in the regulation of the motility of the seminiferous tubules in association with the c-kit positive cells.

Figs. 3 to the 7 show the cellularity of the different compartments of the testicle, and the identification of specific cellular types according to the primary antibodies used in the immunohistochemical techniques.

In Fig. 3, the testicular histology with Papanicolau stain is observed. The staining of the nuclei of the cells of Sertoli and the line of the spermatogenesis of the tubular compartment is outstanding. The peritubular cells are observed of blue color with extended and basophilic nuclei.

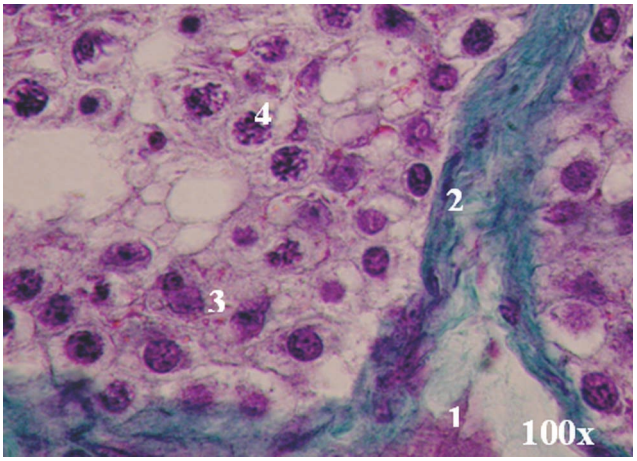

Fig. 3. Histological organization of the adult human testicle with stain of Papanicolau. Peritubular compartment with blue dyeing and the nuclei of the different cellular populations from the tubular compartment, germinal cells and Sertoli strongly basofiles. 1) Interstitial compartment, 2) Peritubular compartment, 3) cells of Sertoli, 4) cells of the germinal line (100x).

Fig. 4 shows the cells of the peritubular compartment using the technique of immunohistochemestry with specific antibodies for actin smooth muscular specific. The samples revealed with AEC allows to observe the positive cells with intense red colour.

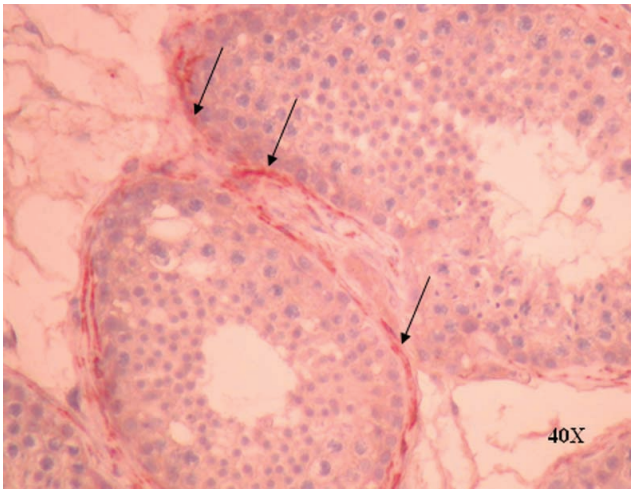

Fig. 4. Transversal section of adult human testicle with immunopositives cells for actin smooth muscular specific. The arrows indicate the positive cells of the peritubular compartment (40x).

Fig. 5. shows positive nervous fibers for specific neurofilaments of the nervous system, which are observed of red color (revealed with AEC) and located towards the vascular tunic of the testicle.

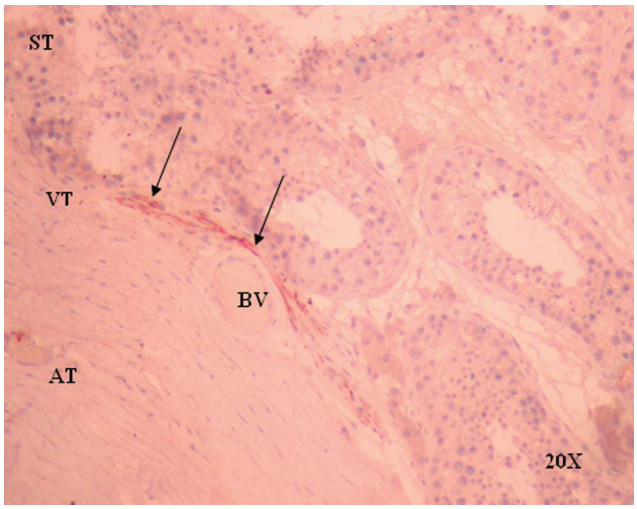

Fig. 5. Transversal section of adult human testicle with immunopositives cells for neurofilaments. The arrows indicate positive nervous fibers, those that are arranged exclusively from the vascular tunic outwards of the testicle (20x). ST: seminiferous tubules; VT: vascular tunic, AT: albuginean tunic, BV: blood vessel. Arrows show nervous fibers (positive neurofilaments).

Fig. 6. shows the cellular populations of the testicle that express the androgen receptor. Cells of Sertoli are observed positive presence of androgen receptor, whereas the germinal line is negative. In the peritubular compartment the positive cells are arranged circularly and in several layers. In the interstitial compartment the positive cells are distributed at random.

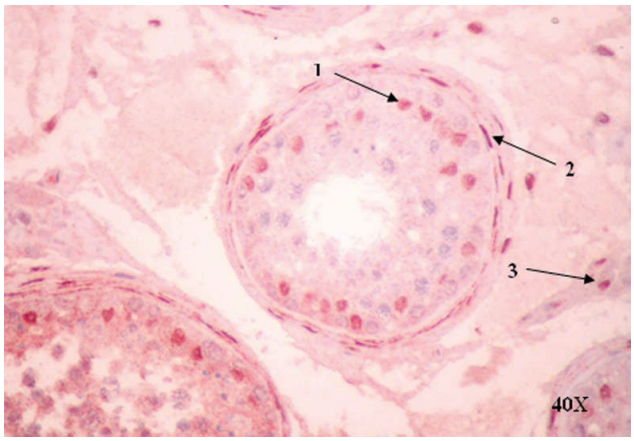

Fig. 6. Transversal section of adult human testicle with immunopositives cells for the androgen receptor (AR). The arrows indicate the nucleus of the immunopositives cells (40x). 1) Tubular compartment; 2) peritubular compartment; 3) interstitial compartment.

Fig. 7 shows the specific immunohistochemical reaction to identify c-kit cells in cross sections of seminiferous tubules. Interstitial cells of Cajal (ICC), located in the interstitium, exhibit a strong immune reaction to the c-kit in their cytoplasm and distributed in the peri-nuclear area. The nucleus of ICC is clear and is surrounded by a clear reddish halo. The ICC are distributed close to the periphery of the seminiferous tubules, in contact with the peritubular cells and separated from the groups of Leydig cells. It must be stated that the tubular and peritubular areas are negative to the c-kit reaction.

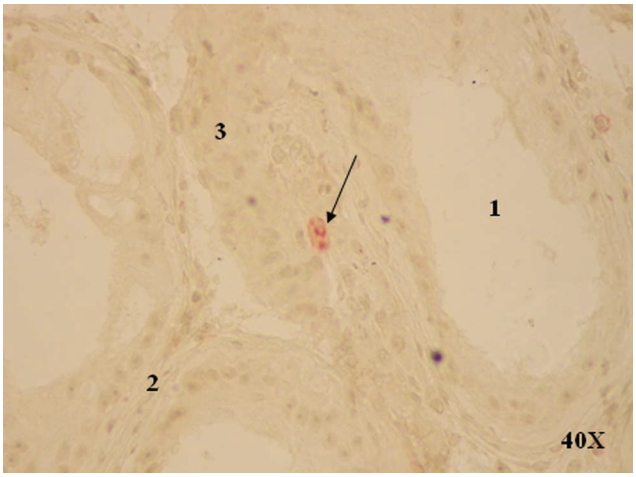

Fig. 7. Sections of human adult testis with immuno-histochemical reaction for the interstitial Cajal cells. The arrow indicate positive reaction for the c-kit receptor in the interstitial Cajal cells, (1) tubular compartment, (2) peritubular compartment, and (3) interstitial compartment (40x).

DISCUSSION

The seminiferous tubules in human are constituted by the seminiferous epithelium, a complex stratified epithelial tissue formed by Sertoli cells and spermatogenic cells. The spermatogenesis includes all the changes that experience the germinal cells: mitosis, meiosis, and cellular differentiation (Tourtellotte *et al*., 1999).

In the seminiferous tubules different cellular associations are identified and represent different stages from differentiation of the germinal cells (Fig. 1). Therefore, in the cross sections of the testicle the adjacent seminiferous tubules display different cellular aspects. This complex cells associations may well be regulated by c-kit cells, as it has been found in the intestinal tract.

In the majority of mammals, the Leydig cells are distributed in the intertubular spaces and are similar in both

ultrastructure and hormonal activity, showing characteristic patterns of growth and development during the fetal and puberty phases, and also secrete variable amounts of testosterone (Bustos-Obregón *et al.,* 2007).

At this time the new molecular concepts revolutionize the medical and clinical practice, with key molecular findings in the pathogenesis and therapeutics of some diseases. A clear example of it corresponds to the cells that display the c-kit membrane receptors. The c-kit cells are originated from mesenchymal stem cells that give origin to the so called ICC (Yorke *et al.,* 2003), also known as pacemaker in the gastrointestinal tract, where they regulate the tubular motility (Hagger *et al.*, 1997).

In the gastrointestinal tract the ICC are located near the mesenteric plexus or distributed separately in the interstice, displaying multiple cytoplasm processes (Park *et al.,* 2004). This is in agreement with our findings, where the c-kit cells are distributed in the interstitial compartment (Fig. 5), near the peritubule (muscle cells, Fig. 2), of the adult human testicle. About a 70% of gastrointestinal mesenchimatic tumors corresponds to mutations in c-kit. These tumors respond very well to therapy with c-kit inhibitors drugs (Joensuu *et al*., 2002). Some of these specific drugs could be useful for future studies of the function of c-kit in the testicle.

The development of the normal testicle depends on the proliferation of primordial germinal cells and their aggregation with the Sertoli cells precursors. All these events are associated to the expression of proto-oncogene c-kit that codifies the protein kinase III (Roskoski, 2005).

Albanesi *et al*. (1996), describe that type A spermatogonia expresses the c-kit receptor in the testicle after birth, and that transcription stops during meiosis, where the proto-oncogene would be active for a very short period at the end of spermatogenesis. In contrast, less information has appeared for c-kit expression in cells of the adult testicle (Bedell & Mahakali, 2004). The deregulation of c-kit has been involved in the genesis of a variety of tumoral pathologies; its role in the pathogenesis of tumors is still not clear (Fine *et al*., 2007). In these circumstances it is possible that the c-kit cells appear increased in number in the testicular interstice, similarly to the situation described in the liver in cases of biliary obstruction and in recent study of pineal gland (Qin *et al*., 2004; Rodríguez *et al*., 2007).

According to the results observed, motility, secretion and cell proliferation might be under c-kit regulation. This would represent the formation of true testicular pacemarkers for the regulation of the motility of the seminiferous tubules and presumably in the fertility of the adult man. All these immunohistochemical aspects here analysed must be considered in future studies of male reproductive organs in human and others models species (Espinoza-Navarro & Bustos-Obregón, 2005; Holt *et al.*, 2004; Espinoza-Navarro *et al.,* 2007).

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RESUMEN: El objetivo de este trabajo fue identificar la presencia de células interticiales de Cajal, células musculares lisas, células nerviosas y células que expresan receptores de andrógeno en el testículo de humano adulto, usando inmunohistoquímica específica para: c-kit/CD-117, músculo liso actina específico (ASMS), neurofilamentos (N) y para receptores de andrógenos (AR). Las muestras fueron obtenidas de pacientes (n=10) con diagnóstico de cáncer prostático sometidos a cirugía de orquiectomía. Las biopsias se procesaron para histología e inmunohistoquímica usando anticuerpos específicos. Se muestra la presencia de células c-kit/CD-117, con diversos grados de positividad y distribuidas en el compartimento interticial del testículo. Las células peritubulares mioides fueron positivas para la presencia de músculo liso actina específico y para receptor de andrógenos. La marcación de neurofilamentos positivos, sólo fueron observados en la túnica vascular. Conclusiones: La inmunohistoquímica específica describe la presencia de células interticiales de Cajal en los interticios testiculares humanos, abriendo una nueva visión en la interpretación funcional de la celularidad testicular y la motilidad tubular. Lo anterior asociado a la funcionalidad de las células peritubulares (músculo liso) regularían la motilidad de los túbulos seminíferos. Este proceso posiblemente es andrógeno dependiente. Las células que expresan receptores c-kit se encuentran exclusivamente en los compartimentos interticiales, estas células en conjunto con las células musculares peritubulares agregado a la ausencia de fibras nerviosas al interior del testículo, podrían ser los responsables de la regulación de la motilidad tubular, similar a como se informa para el tracto gastrointestinal.

PALABRAS CLAVE: Reproducción humana; Testículo; Células interticiales de Cajal; Motilidad tubular; Receptores de andrógenos.

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