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


SUMMARY: The objective of this work was to 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 myoid cells were positive to the presence of 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 peritubulars 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 immunopexpression was observed in Sertoli cells, peritubular myoid cells, Leydig cells, and periarteriolar cells, but not in germinal cells. There
were asked to authorize the use of the tissues, after explaining corresponding to an androgen ablation therapy. The patients of uni or bilateral testicular surgery (orchiectomy) human tissues, according to the Clinical Hospital José Joaquín Aguirre, of the University of Chile, authorized this study.

### 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 anti-human 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 9010 - 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 (Diaminobenzidine) as chromogen (DAB) to develop the color reaction. Endogenous peroxidase was inactivated by 3 % H₂O₂ 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 Megapixels 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 immunopositive 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 pacemaker 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.
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.

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.

<table>
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<th>Cells</th>
<th>Distribution (%)</th>
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<tr>
<td>Cell/ 0.25mm²</td>
<td>Peritubular</td>
</tr>
<tr>
<td>c-kit (+) cells</td>
<td>0.4 ± 0.002</td>
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In Fig. 3, the testicular histology with Papanicolaou 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 basophile nuclei.

In Fig. 4, the transversal section of adult human testicle with immunopositive cells for actin smooth muscular specific. The samples revealed with AEC allows to observe the positive cells with intense red colour.
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. 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. 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.

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...
Ausencia de fibras nerviosas al interior del testículo informa para el tracto gastrointestinal, exclusivamente en los compartimentos interticiales, estas células presentan diversos grados de positividad y distribuidas en el compartimento interticial del testículo. Motilidad tubular. Lo anterior asociado a la funcionalidad de las células de Cajal en los interticios testiculares humanos, abriendo una nueva visión para el tracto gastrointestinal. 

Albanesi et al. (1996), describe que tipo A spermatogonía expresa el c-kit receptor en el testículo después del nacimiento, y que la expresión de c-kit sería activa por un período corto durante la meiosis, donde el proto-oncogene se mantendría inactivo. En contraste, menos información ha aparecido para la expresión de c-kit en células del tracto gastrointestinal (Bedell & Mahakali, 2004). La deregulación de c-kit ha sido observada en casos de obstrucción biliar y estudios recientes del pineal gland (O'Gara et al., 2004; Rodríguez et al., 2007).

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REFERENCES


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