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The Role of α-Smooth Muscle Actin (α-SMA) and Desmin in Human Testicular Peritubular Matrix

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The peritubular matrix has a great significance in endothelial organization during the process of testicular angiogenesis. As a response endotelial cells migrate into interstitium and secrete new matrix components for basal membrane of the seminiferous tubules. The aim of the study was to examine the changes in peritubular matrix under pathological conditions and to investigate localization and distribution of α-SMA and desmin, both are cytoskeletal proteins characteristic for smooth muscle cells. Materials were obtained from testicular biopsies of 9 infertile men and immunohistochemical techniques were applied using monoclonal antibodies against α-SMA and desmin. The results showed positive immunostaining for α-SMA and desmin in peritubular myoid cells and around small blood vessels in interstitium. Our data brings additional support to the view that testicular peritubular matrix proteins play key role in endothelial organization especially in process of angiogenesis and testicular microvasculature is important for normal spermatogenesis.

Key words: peritubular matrix, immunohistochemistry, α-SMA, desmin.

Introduction

The peritubular matrix has a great significance in endothelial organization during the process of testicular angiogenesis. As a response endothelial cells migrate into testicular interstitium and secrete new matrix components for basal membrane of the seminiferous tubules. Testicular peritubular and interstitial tissues interact in peritubular space and the basic components of this space that are vulnerable during pathological conditions are: small blood vessels, Leydig cells, macrophages and peripheric nerves [4].

In patients with severe testicular lesions in cases with azoospermia hyalinization process often occurred in small blood vessels and around seminiferous tubules. In patients with oligoastenozoospermia a complete tubular hyalinization was demonstrated [10]. According to the authors the hyalinization affects arterioles and venules, but not capillaries. Testicular small blood vessels revealed altered structure — they have narrow lumen and thick blood vessel's wall. This finding is supported by our previous examinations [6].

Harindel and Trainen [3] accented on testicular microvasculature, mainly because of the fact that it is modulator of endocrine and paracrine factors that are important in regulation of spermatogenesis. In turn, Leydig cells play a key role in regulation of blood transport.

Peritubular matrix is responsible for the function of endothelial cells and is also important for proliferation of these cells. On the other hand, matrix components are linked to intracellular structural and contractile filaments forming an interacting functional unit that is of great importance for testicular morphogenesis. In this respect the aim of present study was to examine: 1) the morphological changes in peritubular matrix under pathological conditions and 2) localization and distribution of desmin as a marker for peritubular myofibroblasts and of α-SMA — for terminal differentiation of vascular smooth muscle cells.

Material and Methods

Materials were obtained from testicular biopsies of 9 infertile men. In two of them the semen analysis showed oligoastenozoospermia III degree and in four — azoospermia on the base of pseudocrypthorchidism billateralis. In three men oligoastenozoospermia I degree is observed without any morphological alterations and they served as controls as usual.

Testicular biopsies for light microscopy (morphological and immunohistochemical examination) were fixed in Bouin's, dehydrated and embedded in paraffin. Sections (7μm) were processed according to the classical immunochemical techniques using monoclonal antibodies against desmin and α-SMA. Avidin-biotin-peroxidase method was applied and histostain kit from DAKO was used. Immunofluorescence for α-SMA was also performed. After the incubation with mouse anti-α-SMA the sections were incubated with FITC conjugated goat mouse antibody. The sections were mounted with Moviol and observed at POLYVAR immunofluorescent microscope.

Results

In cases of azoospermia disorganization of germinal epithelium was evident and basal membrane was folded at some places or destructed at others (Fig. 1). The results from immunohistochemical study showed positive reaction for desmin in basal membrane and peritubular myofibroblasts, as well. In cases of azoospermia and oligostenozoospermia III degree desmin was localized as dots or has fibrilar appearance and it was not homogeniously distributed in comparison with other matrix proteins (Fig. 1 and Fig. 2). Very strong immune reaction for desmin was also observed around blood vessels (Fig. 3).

Strong intensity of immune reaction of α-SMA was found in the testis in cases of oligoastenozoospermia. Strong reaction was observed in basal membrane of the seminiferous tubules and peritubular myofibroblasts. In basal membrane α-SMA was localized mainly in inner layer (Fig. 4). Strong immunoreactivity for α-SMA was found around small blood vessels in interstitium (Fig. 5).

The results form immunofluorescence confirm our immunohistochemical findings and showed very strong immune reaction in basal membrane of the seminiferous tubules, in peritubular and interstitial tissues, around Leydig cells and small blood vessels (Fig. 6).

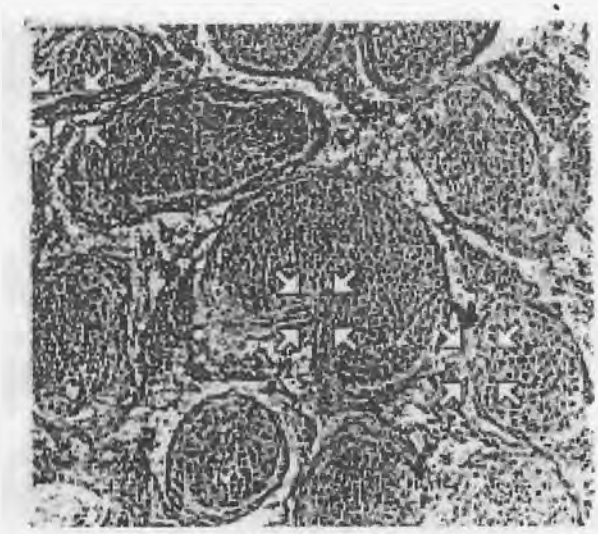

Fig. 1. Testicular biopsy. Azoospermia. Desorganization of germ epithelium. The basal membrane at some places is folded, at other is destructive (arrows). Desmin is localized in some places in basal membranes as dots or fine fibrils. Monoclonal anti-desmin antibody (× 200)

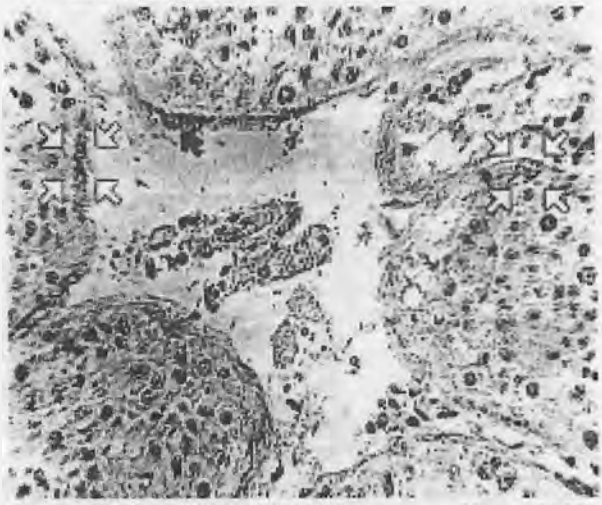

Fig. 2. Testicular biopsy. Oligoastenozoospermia III dg. Immune reaction for desmin, showing it's localozation as dots, and its fibrilar appearance, respectively. Monoclonal anti-desmin antibody (× 400)

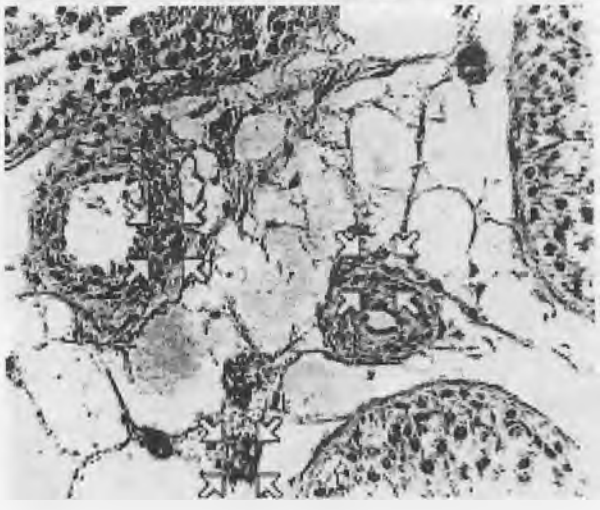

Fig. 3. Testicular biopsy. Oligoastenozoospermia III dg. Possitive immune reaction for desmin, showing it's localization around blood vessels. Monoclonal anti-dsmin antibody (× 400)

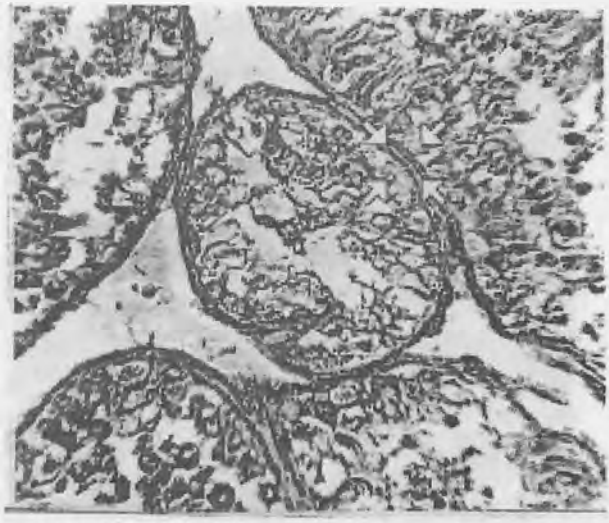

Fig. 4. Testicular biopsy. Oligo-astenozoospermia III dg. Strong immune reaction for α-SMA, showing it's localization in basal membranes of seminiferous tubules and in peritubular myofibroblasts. Monoclonal anti- α-SMA antibody (× 200)

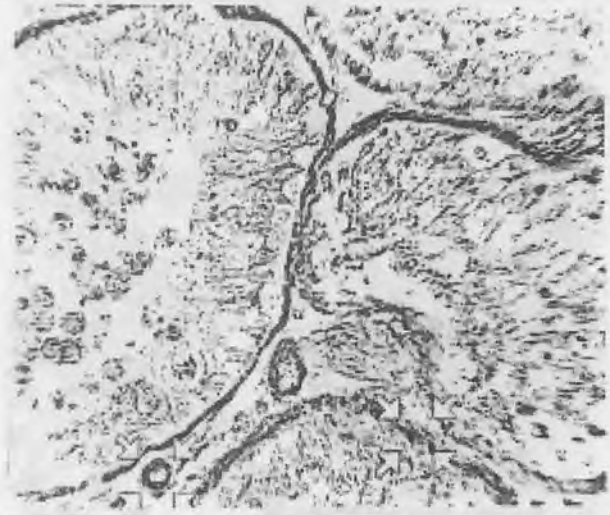

Fig. 5. Testicular biopsy. Oligo-astenozoospermia III dg. Strong immune reaction for α-SMA around blood vessels in interstitium. Monoclonal anti-α-SMA antibody (× 400)

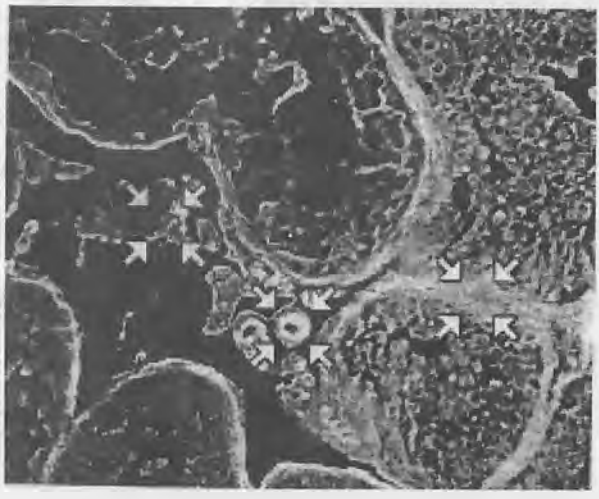

Fig. 6. Testicular biopsy. Oligoastenozoospermia III dg. Direct immunofluorescence for α-SMA. Strong immune reaction is observed in basal membranes of seminiferous tubules, in testicular interstitium, around Leydig cells and small blood vessels (× 200)

Discussion

Peritubular myofibroblasts produce α-SMA that is important for differentiation of vascular smooth muscle cells and myofibroblasts themselves are involved in cell to cell and cell to matrix interactions. All this is important for testicular morphogenesis [7, 8, 11]. Our results showed that myofibroblasts expressed α-SMA and desmin both are cytosceletal proteins characteristic for smooth muscle cells. It was reported that most myofibroblasts of the thickened lamina propria (as a result of pathological processes) lost their ability to express desmin suggesting that they may change their phenotype [1,2,5]. That was supported by Skalli [9] and Virtanen, Kollajoki [12]. In these circumstances we found that less myofibroblasts were immunoreactive for desmin. They were individual cells and are arranged in uncomplete, discontinuous layer.

Small blood vessels in testicular interstitium are this part of vascular system in testis that is responsible for the exchange of gases, nutritional substances and hormones [3] and is responsible for endocrine and paracrine regulation and normal testicular function.

In conclusion, our data brings additional support to the view that testicular peritubular matrix proteins play key role in endothelial organization especially in process of angiogenesis and testicular microvasculature is important for normal spermatogenesis.

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