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# Immunohistochemical Distribution of Insulin-Like Growth II (IGF II) and Its Receptor in the Human Testis

*Y. Koeva*

*Department of Histology and Embryology, Medical University, Plovdiv*

The aim of the present study was to show the immunohistochemical pattern of distribution of insulin-like growth factor II (IGF II) and its receptor, IGF II receptor in the human testis. IGF II and its receptor were localized by means of an amplification immunocytochemical technique in the Leydig cells of the human testis. Within seminiferous tubules, the Sertoli cells and some of the germ cells (primary spermatocytes) also exhibited moderate to strong immunoreactivity for the examined antigens. Our results providing immunocytochemical evidence for the existence of IGF II and its specific receptor in the human Leydig cell (also in other cell components) indicate that the growth factors may play an auto-and/or paracrine role in the local control of the testicular functions.

*Key words*: IGF II - IGF II receptor - Leydig cells — human testis

## Introduction

Numerous growth factors have been reported to be produced and/or expressed by different testicular cell types, including the Leydig cells [1, 3] suggesting that these factors, locally produced in testis, are regulatory proteins involved in modulating testicular functions via auto- and/or paracrine modes [3, 9]. IGF I and IGF II are anabolic polypeptides with structural homology to proinsulin (6, 7) and their effect is mediated through the specific cell membrane receptors. IGF II acts as growth and differentiation promoting factor during the period of embryogenesis [2]. IGF I act by binding to the IGF I receptor that is heterotetrameric complex with tyrosine-protein kinase activity [5]. IGF II receptor binds IGF II with higher affinity than IGF I and does not possess tyrosine-kinase activity. The role of IGF II receptor is still unclear and the effects of IGF I and II appear to be predominantly mediated through the IGF I receptor [5]. To date, data about the existence and possible role of IGF II in the regulation of Leydig cell functions are still lacking.

In context with the potential role of growth factors in modulating testicular functions, the present study was designed to establish the localization of IGF II and its corresponding receptor in human testis by immunohistochemistry.

## Material and Methods

### Immunohistochemistry

Testes were obtained from patients ($n$=6) aged between 54-86 years who had undergone orchidectomy for treatment of a prostate carcinoma. Blocks approximately 4-5 mm thick were fixed in Bouin's fluid for 24 hours and embedded in paraffin. For the detection of the antigens an amplification technique consisting of the combination of the peroxidase anti-peroxidase (PAP) and the avidin-biotin-peroxidase complex (ABC) methods was applied [4]. The paraffin wax sections were incubated for 24 hours at 4 °C with specific primary antibodies, as follows: 1. monoclonal mouse **anti- IGF II antibody** (Serotec, UK; 1:40); 2. polyclonal rabbit **anti- IGF II receptor antibody** (Serotec, UK; 1:200). In the next steps biotinylated anti-rabbit and anti-mouse IgG in final dilution 1:250 (Dakopatts, Denmark), rabbit and mouse PAP (Dakopatts, Denmark, diluted 1:100) and ABC (Vector, USA, in dilution 1:250) were applied. The peroxidase activity was then developed by means of the nickel-gluckoseoxidase technique [10].

*As controls,* sections were used in which the primary, secondary or tertiary antibodies were replaced by phosphate-buffered saline (PBS) or only the peroxidase activity was visualized.

## Results

Strong IGF II- immunoreactivity was observed in the interstitial and peritubular Leydig cells of human testis with differences in the staining intensity between individual Leydig cell in a group, as well as between adjacent Leydig cell clusters. Mod-


Fig. 1. Strong IGF II- immunoreactivity in a group of Leydig cells (a). Positive were also the Sertoli cells predominantly in their basal part (b) and primary spermatocytes (c) (× 820)

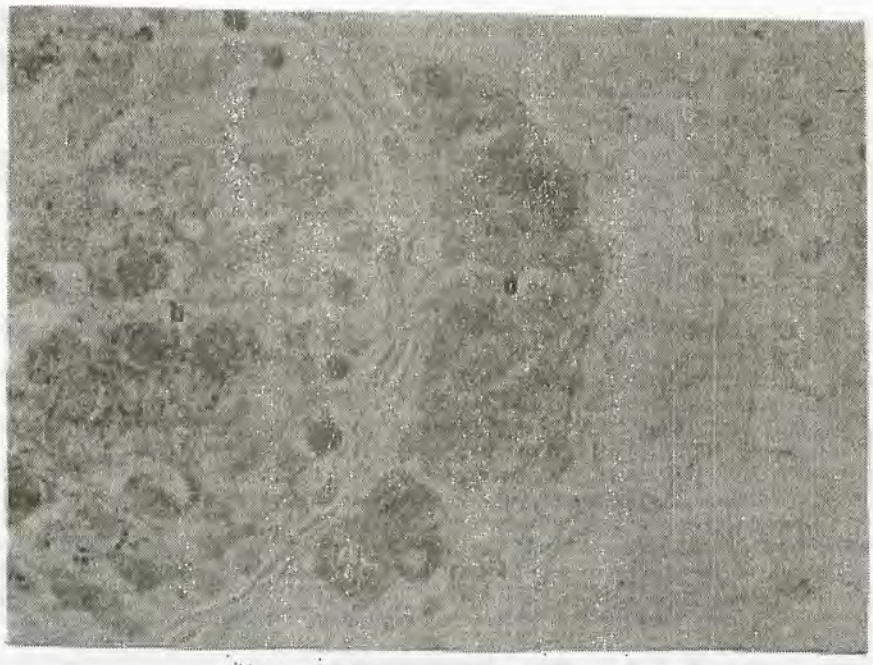

Fig. 2. Moderate IGF II receptor-staining intensity in the Leydig cells (a). Positive reaction also in primary spermatocytes (b) (× 820)

erate immunostaining for IGF II showed also the Sertoli cells and primary spermatocytes (Fig. 1). Immunoreactivity for IGF II receptor of moderate staining intensity was observed in the Leydig cells, whereas primary spermatocytes and early spermatids were strong immunopositive. The reaction product was with cytoplasmic localization. The Sertoli cells showed a weak staining for IGF II receptor (Fig. 2). No specific immunostaining was found in the control sections (not shown).

## Discussion

In this study we have demonstrated that the human Leydig cells exhibited strong IGF II immunoreactivity with individual differences of staining intensity. Positive for the examined antigen were also the Sertoli cells and primary spermatocytes. The observed heterogeneity in the staining intensity between individual Leydig cells in a group, as well as between neighbour Leydig cell clusters, could be referred to the existence of different Leydig cell populations in adult testis (8). Moderate IGF II receptor staining intensity was found in the Leydig cells, whereas primary spermatocytes and early spermatids were strong positive. Different to our results, it has been reported that IGF II mRNA was identified by in situ hybridization in the endothelium and adventitia of testicular blood vessels and in the peritubular cells but was not localized in the Leydig and germ cells of human testis [11]. IGF II receptor mRNA was mainly concentrated in the primary spermatocytes, while little receptor mRNA was found in more mature spermatocytes and in spermatids [11]. The lack of concordance between the above data and our immunocytochemical findings, exclusively with respect to the IGF II mRNA localization, could be explained by a fact that the peptides are not stored inside the cells in which they are synthesized. Probably in situ hybridization is ineffective to detect the small amounts of IGF II mRNA in human Leydig cells.

Our findings demonstrating the immunocytochemical distribution of IGF II and its corresponding receptor in the human Leydig cells (also in the Sertoli and some germ cells) suggest that the growth factors may play a significant role in the local auto/paracrine control of Leydig cell functional activity.

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