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Immunohistochemical demonstration of LH/CG receptors in non-neoplastic human adrenal cortex and adrenocortical tumors

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Abstract
Introduction. Numerous data indicate that luteinizing hormone and/or chorionic gonadotropin (LH/CG) exert direct actions on the adrenal cortex and are involved in the adrenal pathology. However, the immunohistochemical studies on the expression of LH/CG receptors (LH/CGR) in the human adrenal cortex and in the adrenocortical tumors are scarce.
Material and methods. Paraffin sections of samples of 6 human non-neoplastic adrenal cortex and 25 adrenocortical tumors were immunostained with anti-LH/CGR polyclonal antibody.
Results. All zones of the human non-neoplastic adrenal cortex present a positive immunoreaction with anti-LH/CGR antibody showing the strongest reaction in cell membranes. The LH/CGR immunostaining in the vast majority of hormonally non-functioning adenomas and in all hormone-secreting adenomas does not differ from the non-neoplastic adrenal cortex. In contrast to non-neoplastic adrenal cortex and benign adenomas, in adrenocortical cancers the immunostaining with anti-LH/CGR antibody behaves differently. The immunopositive material is almost totally filling the cytoplasm of the cells but the immunopositivity of cell membranes is weak or lacking.
Conclusions. The data presented in our study show that the expression of LH/CGR in adrenocortical tumors is not ectopic but eutopic. The immunohistochemical examination of LH/CGR may be useful in the differentiation between benign and malignant lesions in the adrenal cortex. Moreover, the loss of membrane localization of LH/CGR in adrenocortical cancer suggests the alteration of receptors' function. *(Folia Histochemica et Cytobiologica 2019, Vol. 57, No. 1, 23–27)*

Key words: adrenal cortex; adrenocortical adenomas; adrenocortical cancer; luteinizing hormone/chorionic gonadotropin receptor

Introduction

The data indicating the direct actions of gonadotropins on the adrenal gland were published as early as in the sixties of the 20th century [1–3]. The quoted authors observed the morphological changes in the adrenal cortex of the rat under the influence of exogenous administration of LH and/or FSH. Since the model of gonadectomized-hypophysectomized animals was used in the experiments, the mediation of gonadal steroids could be excluded.

In the forthcoming years the gonadotropin receptors' structure was elucidated as membrane G-protein-coupled elements possessing seven transmembrane domains [4]. The further studies showed the presence of lutropin/chorionic gonadotropins receptors (LH/CGR) in the normal human adrenal cortex [5, 6]

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Table 1. The source of adrenocortical samples included in the study

Histopathological diagnosis	Clinical status	No of patients
Normal adrenal cortex	No alterations of the adrenal function	3
Adrenocortical hyperplasia	No alterations of the adrenal function	3
Adrenocortical adenoma	Non-functioning tumor	11
Adrenocortical adenoma	Conn's syndrome	5
Adrenocortical adenoma	Cushing's syndrome	4
Adrenocortical cancer	Malignant tumor, Cushing's syndrome	1
Adrenocortical cancer	Non-functioning malignant tumor	4

in human adrenocortical aldosterone-secreting [6–8] or cortisol-secreting adenomas [9–10] and adrenocortical cancers [11]. The functional LH/CGR were also found in human adrenocortical cancer cell line H 295R cells [12]. The data on LH/CGR in adrenal tissues were collected mostly using molecular biology techniques [6–11] but no systemic immunohistochemical studies of adrenocortical tumors and non–tumoral human adrenal cortex are available.

Material and methods

Samples of adrenal glands. The archival material of 25 surgically excised adrenal tumors stored in paraffin blocks was studied. The detailed presentation of material was shown in Table 1. In addition 6 non-tumoral adrenal glands were studied. Two of them were removed from patients suffering from renal cancers during the surgical excision of the affected kidney, one was excised together with pheochromocytoma tumor, and 3 were diagnosed as adrenocortical hyperplasia. The study was approved by the Ethical Committee of the Medical University of Lodz, decision RNN/335/17/KE dated 21 November 2017.

Immunohistochemistry. Paraffin sections were immunostained with anti-LH/CGR polyclonal antibody PA 1552 (Boster Biologicals Technologies, Pleasanton, CA, USA). This antibody binds the N-terminal part of LH/CGR (127-143 aa). The slides were incubated with antibody (1:150) for 24 h at 4°C The visualization of immunostaining was performed using Envision kit (DAKO, Glostrup, Denmark) with the use of 3,3' diaminobenzidine (DAB) as chromogen. For a positive control, a biopsy sample of the human testis was immunostained (Fig. 1A). For a negative control, the primary antibody was omitted in the immunostaining procedure.

Statistical analysis. Since we present only qualitative descriptions no statistical analysis was performed.

Results

All zones of the human non-neoplastic adrenal cortex show a positive immunoreaction with anti-LH/CGR antibody. In zona glomerulosa and zona fasciculata the strongest immunoreaction is visible in cell membranes (Fig. 1B and C). On the other hand, the cytoplasmic area of adrenocortical cells is mostly empty because of the washing out of the fat deposits. A scarce residual immunostaining was also present. In zona reticularis the distribution of immunoreaction is similar but a part of cells contain the abundant immunoreactive material in their cytoplasm (Fig. 1D).

The LH/CGR immunostaining in the vast majority (10/11) of hormonally non-functioning adenomas and in all cortisol-secreting adenomas does not differ from the non-neoplastic glomerulosa and fasciculata zones (Fig. 2A and B). In one case of non-functioning adenoma the loss of immunoreaction within cell membranes and homogenous immunoreaction of cytoplasm was noticed. In aldosterone-producing adenomas (Conn's syndrome) the immunostaining of cell membranes does not differ from normal zona glomerulosa, but the density of the immunoreaction of cytoplasm is more variable (Fig. 2C).

In contrast to non-neoplastic adrenal cortex and benign adenomas, in adrenocortical cancers the immunostaining with anti-LH/CGR antibody behaves differently. The immunopositive material of variable intensity is almost totally filling the cell cytoplasm. In contrast, the immunopositivity of cell membranes is very weak or lacking except one case (Fig. 2D).

Discussion

The expression of LH/CGR in non-neoplastic adrenal cortex and in certain adrenal tumors is well established in many studies conducted with molecular

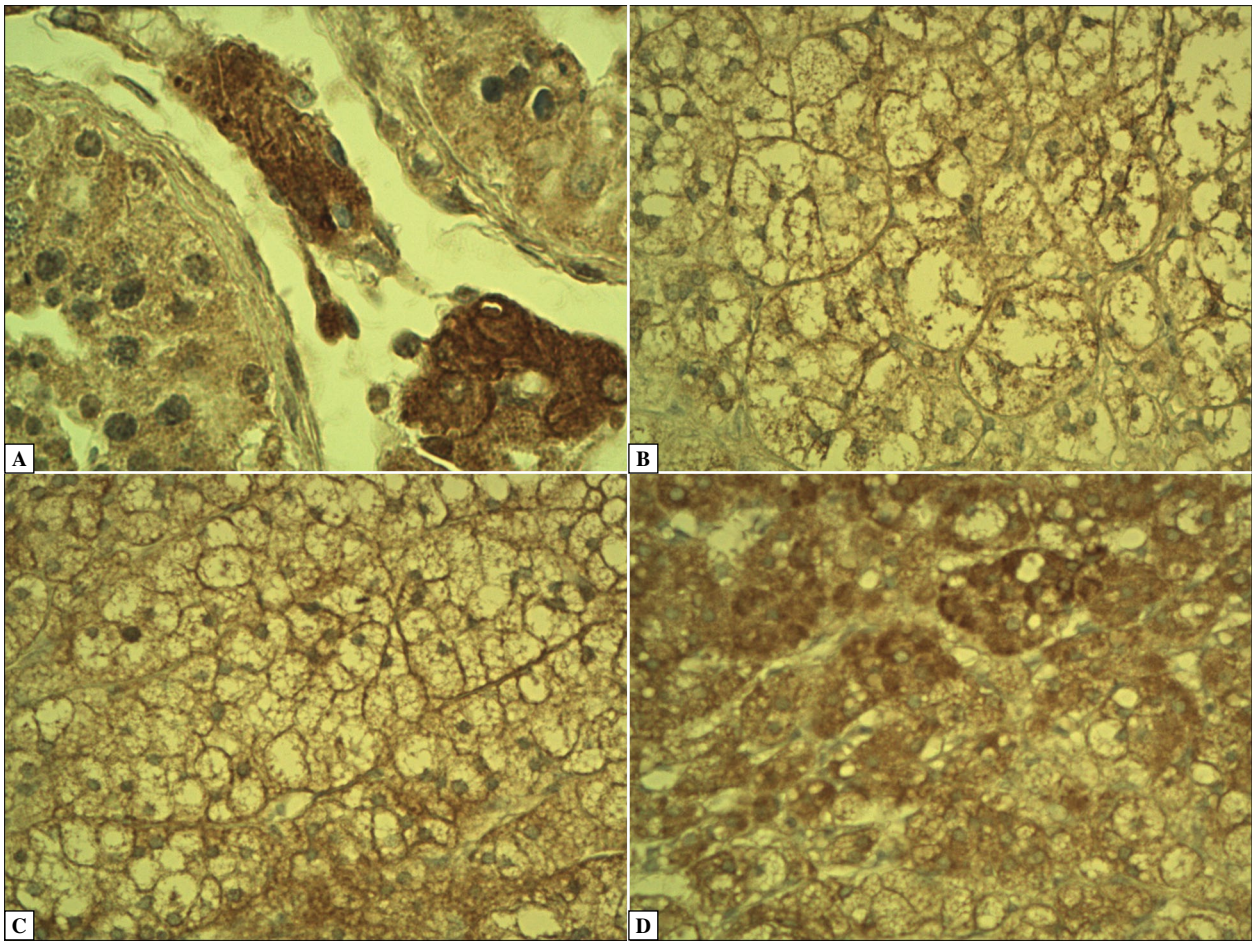

Figure 1. Immunostaining of LH/CGR in human non-neoplastic adrenocortical samples and testis. **A.** Positive control, human Leydig cells, 400×. **B.** Non-neoplastic adrenal cortex, glomerular zone, 200×. **C.** Non-neoplastic adrenal cortex, fascicular zone, 200×. **D.** Non-neoplastic adrenal cortex, reticular zone, 200×.

biology techniques [4–13]. Although these studies present a high level of credibility, the information on precise tissue localization of LH/CGR is insufficient. Such data could be only supplied by morphological methods, mainly by immunohistochemistry. However, the immunohistochemical studies on the localization of LH/CGR in human adrenal cortex and adrenal tumors are very scarce. Lesley *et al*. [14] investigated the presence of LH/CGR in the normal adrenal cortex of Rhesus macaque. They found, like we did in the humans, the positive immunoreactivity for LH/CGR in all adrenocortical zones. It is also worth recalling that we found similar immunopositivity in the normal adrenal cortex and adrenal tumors using the antibody for follicle stimulating hormone receptors (FSHRs) [15].

The LH/CGR immunopositivity which we found in adrenal benign and malignant tumors is in agreement with the results of previous studies [6–11]. Because the LH/CGR expression takes place also in the normal adrenal cortex, there is no reason to call them „ectopic". The sharp difference in the topography of LH/CGR immunoreaction between the benign and malignant adrenal neoplasms is worth to underline. Because of that the immunohistochemical examination of LH/CGR seems to be useful in the differentiation between benign and malignant tumors of the adrenal cortex during the histopathological diagnosis. To our best knowledge, this finding has not been reported previously in the literature. However, our findings need to be confirmed on the larger material. Moreover, since LH/CGR belongs to membrane receptors, the partial or total loss of their membrane localization in adrenocortical cancers suggests the alteration of their function. The described loss of membrane localization may reflect either down-regulation due to decreased expression of receptor gene at transcriptional level or the increased internalization of the receptor protein resulting from the ligand excess. The further studies, especially *in vitro*, concerning these possibilities are needed. The role of the direct action of LH

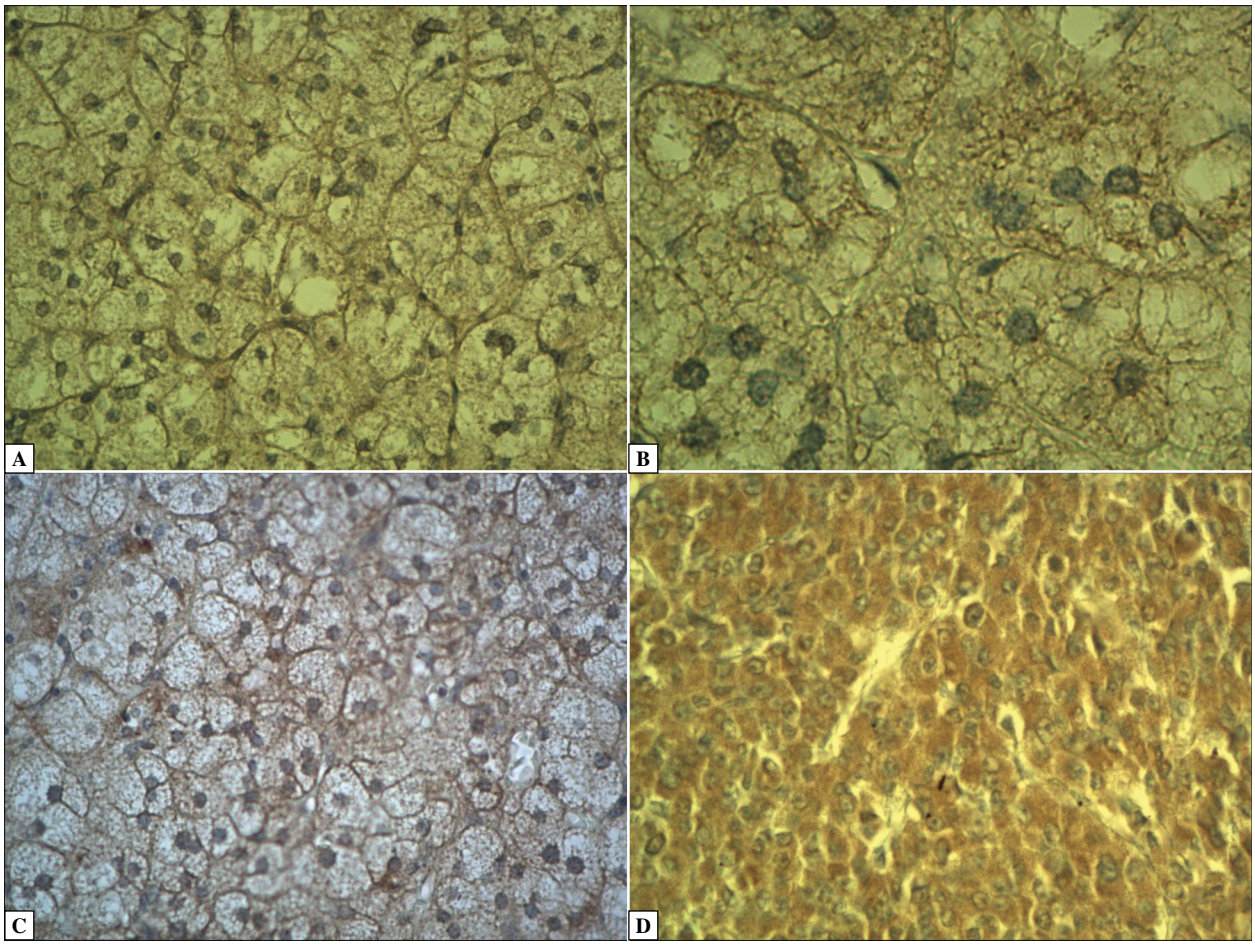

Figure 2. Immunostaining of LH/CGR in human neoplastic adrenocortical samples. **A.** Non-functioning adrenocortical adenoma, 200×. **B.** Cortisol-secreting adrenocortical adenoma (Cushing syndrome), 400×. **C.** Aldosterone-secreting adrenocortical adenoma (Conn's syndrome), 200×. **D.** Hormonally non-functioning adrenocortical cancer, 200×.

in adrenal cortex is not fully understood. It probably acts as an additional regulator of steroidogenesis and adrenocortical cell growth. Its involvement in adrenocortical tumorigenesis has been also suggested and proved on certain animal models [16, 17].

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