Localization of Epidermal Growth Factor (EGF) and Epidermal Growth Factor Receptor (EGFr) in Human Pituitary Adenomas and Nontumorous Pituitaries: An Immunocytochemical Study

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Abstract

Epidermal growth factor (EGF) and epidermal growth factor receptor (EGFr) were investigated by immunocytochemistry (ICH) in 57 human pituitary adenomas and 10 nontumorous autopsy pituitaries. EGF immunoreactivity was demonstrated in 24 adenomas (42%), representing 23 functioning tumors and 1 nonfunctioning tumor of oncocytic type, and in all nontumorous pituitaries. Among 40 tumors, EGFr was found positive in 15 functioning adenomas (37.5%), representing 50% of them. The presence of both EGF and EGFr was found mainly in corticotroph adenomas (60%) and less frequently in somatotroph and lactotroph adenomas (20%). ICH on serial sections with EGF or EGFr and adrenocorticotrophic hormone (ACTH) or S-100 protein revealed that EGF and EGFr are localized specifically in corticotrophs and EGFr in stellate cells of nontumorous adenohypophysis.

These results confirm the presence of EGF and EGFr in human pituitary adenomas and nontumorous pituitaries and highlight their frequent occurrence in hormone-producing adenomas. Further work is required to explore the possibility that EGF and EGFr play a role in hormone production, release, and tumor progression.

Key Words: Growth factors (GFs); receptors; c-*erb* B-1; human pituitary; adenoma; corticotrophs; stellate cells.

Introduction

Growth factors (GFs) constitute a family of polypeptides with growth-promoting properties, which act by autocrine or/and paracrine fashion, contributing to cell-to-cell communication [1]. GFs binding to GF receptors, which represent specific sites of the cytoplasmic membrane, are necessary for biologic activities. GFs are distributed in various tissues and some are present in endocrine glands including pituitary [2–4]. The effects of GFs have been studied primarily in rat pituitaries and tissue cultures [5–10]; several GFs have been demonstrated in human nontumorous and adenomatous pituitaries by immunocytochemistry (ICH) and blotting techniques [11–16].

Epidermal growth factor (EGF) and epidermal growth factor receptor (EGFr), the latter also known as c-*erb* B-1, are structurally well-characterized molecules [17,18]. EGF was found to be involved in regulation of hormone secretion by rat pituitary cells in tissue cultures [6–8,19]. Only limited information is available on the presence and function of EGF and EGFr in pituitary; the sites of their production and expression in human non-

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tumorous pituitaries have not been studied extensively at light and electron microscopic levels. Localization studies of EGF and EGFr have only been performed recently [11,15,20–22].

The aim of this study was to demonstrate by ICH the distribution of EGF and EGFr in human pituitary adenomas, as well as nontumorous glands and to reveal the particular cell types in which they are produced.

Materials and Methods

Fifty-seven surgically removed pituitary adenomas, retrieved from the files of the Departments of Pathology, St. Michael's Hospital, Toronto, Ontario, Canada (40 cases), and General Hospital of Athens, Greece (17 cases), were studied. All tumors were diagnosed and classified by histology, ICH for anterior pituitary hormones, and electron microscopy.

The tumors included 16 somatotroph, 13 lactotroph, 14 corticotroph, and 14 nonfunctioning glycoprotein-producing adenomas. The latter were represented by 5 null cell adenomas, 5 oncocytomas, 2 with signs of glycoprotein differentiation, and 2 exhibiting features of gonadotroph adenoma. Twenty specimens from surgically removed adenomas included fragments of adjacent nontumorous adenohypophysis. In addition, 10 nontumorous autopsy pituitaries from patients with nonendocrine disorders were studied as well.

Antibodies were applied toward the following hormones and glycoprotein hormone subunits: growth hormone (GH), prolactin (PRL), adrenocorticotrophic hormone $(ACTH)_{1-39}$, β-subunit of thyroid-stimulating hormone (TSHβ), β-subunit of follicle-stimulating hormone (FSHβ), β-subunit of luteinizing hormone (LHβ), and β-subunit of human chorionic gonadotropin. The morphologic methods and application protocols for pituitary hormone ICH were previously described in detail [23].

For EGF and EGFr ICH, the labeled streptavidin–biotin–peroxidase-conjugated (LSAB) detection system was employed (Dako, Santa Barbara, CA). Sections were incubated overnight at 4°C with monoclonal EGF antibody (dilution 1:300, Sigma, St. Louis, MO). Before application of EGF antibody, sections were pretreated with 0.16 mg/100 mL trypsin (Sigma) for 15 min at 37°C. All 57 adenomas were immunostained for EGF. Of these, 40 tumors from St. Michael's material, equally represented by somatotroph, lactotroph, corticotroph, and nonfunctioning glycoprotein-producing adenomas (null cell, oncocytic, and gonadotroph), and 10 nontumorous pituitaries as well, were further studied with monoclonal EGFr antibody (dilution 1:150, clone F_4, Sigma), which is specific for the intramembrane part of the receptor. Similar to EGF, incubation of sections with 1 mg/100 mL pronase (Sigma) was carried out before exposure to EGFr antibody. No tissue was available from the remaining adenomas. Normal skin and small bowel mucosa were used as positive controls. To test the specificity of EGF immunoreactivity, the antibody was preabsorbed with equal volume of purified mouse EGF antigen (Sigma) at dilutions ranging from 1:100 to 1:300 by overnight incubation at 4°C. The absorbed antiserum was consequently microfuged for 15 min at 4°C and the supernatant collected for use. Then ICH was applied to pairs of serial sections obtained from both surgical and autopsy pituitary samples. One section of each pair was treated with preabsorbed and the other with nonpreabsorbed EGF antiserum.

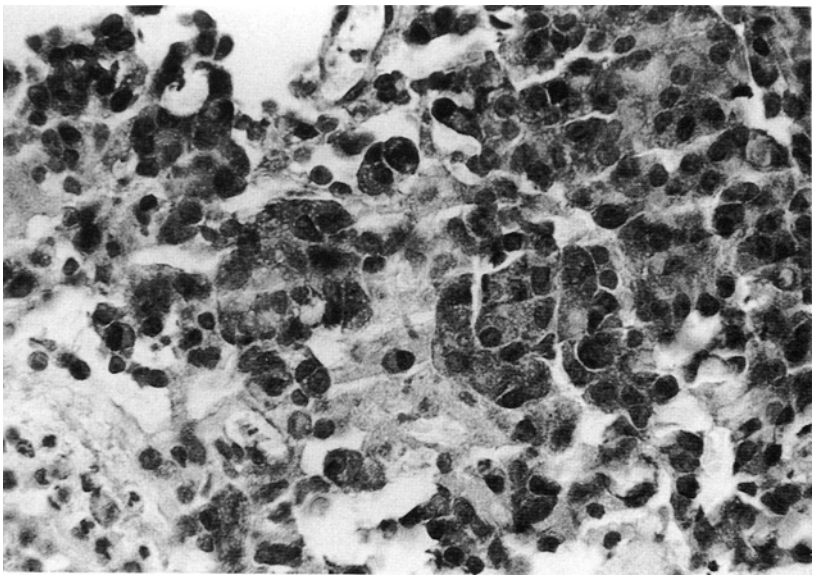

Fig. 1. Immunoreactivity for EGF in a sparsely granulated lactotroph adenoma. The distribution of chromogen is fairly uniform and the density strong (LSAB, ×740).

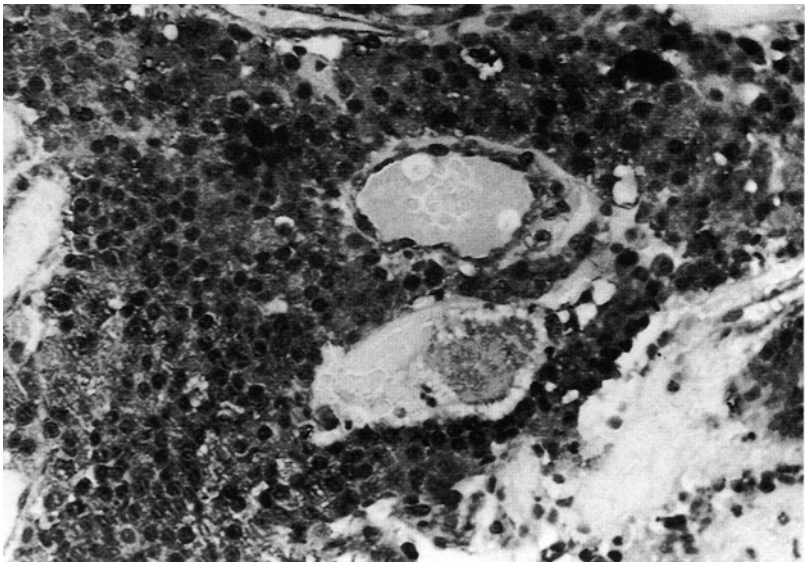

Fig. 2. Strong immunoreactivity for EGF in a corticotroph adenoma (LSAB, ×460).

To confirm the presence of EGF and/or EGFr in corticotrophs and stellate cells, serial sections from autopsy glands were exposed each one of two to EGF, EGFr, and ACTH or S-100 protein (dilution 1:2000, Dako) antisera, respectively. It is well known that S-100 protein is specifically localized in stellate cells of the anterior pituitary lobe.

Results

Immunoreactivity for EGF

EGF immunoreactivities were detected in 24 of 57 adenomas (42%), representing 23 functioning adenomas (53.5%) and 1 oncocytoma among nonfunctioning tumors (7%). The tumors contained a few to moderate numbers of EGF-positive cells disposed in small foci or in large areas with diffuse immunoreactivity, except for one lactotroph and one corticotroph adenoma; in these tumors all cells showed strong immunopositivity for EGF (Figs. 1 and 2). Localization of EGF was intracytoplasmic with a diffuse staining pattern. The specificity of EGF antibody was investigated by ICH on serial sections exposed to EGF antiserum preabsorbed with EGF antigen and nonpreabsorbed EGF antibody. Complete abolishment of immunoreactivity for EGF was found in sections treated with preabsorbed EGF antiserum.

In the anterior lobe of all autopsy glands and surgically removed fragments of nontumorous adenohypophyses, several large polyhedral, periodic acid-Schiff-positive cells, mostly arranged in groups and often containing large paranuclear vacuoles, were consistently EGF positive. In addition, EGF positivity was demonstrated in the cytoplasm of basophilic cells extending to the posterior lobe (basophil invasion) and nontumorous adenohypophysial cells with Crooke's hyaline change, associated with corticotroph adenoma Serial sections revealed that most of the EGF-positive cells immunostained for ACTH corresponded to corticotrophs. In addition to corticotrophs, a few scattered anterior pituitary cells were EGF positive (Figs. 3 and 4). However, no combined immunostains on serial sections with EGF and the remaining pituitary hormones were performed.

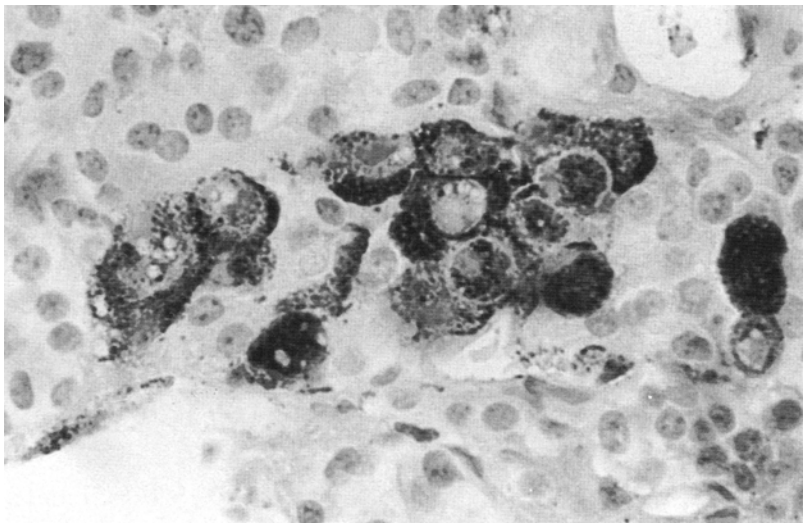

Fig. 3. ACTH immunoreactive corticotrophs exhibiting typical Crooke's hyaline change in nontumorous pituitary adjacent to corticotroph adenoma (LSAB, ×1100).

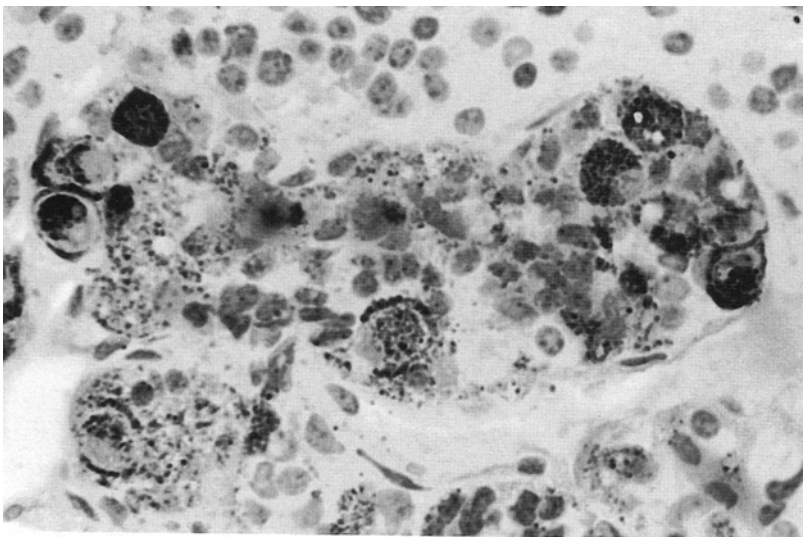

Fig. 4. Similar to Fig. 3 selective localization of EGF in Crooke's cells. In addition to corticotrophs, scattered secreting cells immunoreactive for EGF are demonstrated (LSAB, ×740).

Immunoreactivity for EGFr

Overall, 15 of 40 adenomas (37.5%) representing 50% of functioning tumors were immunopositive for EGFr (Table 1). In contrast, all nonfunctioning glycoprotein-producing adenomas were negative. EGFr-immunoreactivity was found in 70% of somatotroph and 60% of corticotroph adenomas, and less frequently in lactotroph adenomas (20%). In somatotroph and corticotroph adenomas, most positive cells exhibited strong immunoreactivity, comprising >50% of tumor cell population. In contrast, only few and mildly immunoreactive cells were noted in lactotroph adenomas. Correlation between EGF and EGFr was noted only in corticotroph adenomas (60%).

Immunoreactivity for EGFr was heterogeneous, often with focal and rarely diffuse distribution; the degree of immunoreactivity was variable (Fig. 5). Localization of EGFr was mostly intracytoplasmic, with some areas of peripheral immunoprecipitate accumulation along the cytoplasmic membranes (Fig. 6). It should be noted that the degree and density of plasmalemal immunoreactivity was uneven among cells of the same tumor.

In nontumorous pituitaries, EGFr was localized mostly in stellate cells and corticotrophs with or without Crooke's hyaline change, as demonstrated by similar distribution and staining pattern in combined serial sections immunostained for EGFr/S-100 protein and EGFr/ACTH, respectively. Stellate cells characterized by long cytoplasmic processes and S-100 immunoreactivity were scattered among hormone-producing cells and occasionally participated in follicle formation (Fig 7). Additionally, a few scattered anterior pituitary cells were also positive for EGFr (Fig. 8). The posterior lobe exhibited moderate-to-strong immunoreactivity for EGFr in nerve endings and in pituicytes.

Discussion

Most studies regarding EGF and EGFr have focused on their mitogenic properties and participation in neoplastic transformation, as has been shown in several

Table 1. Localization of EGF and EGFr Among 40 Surgically Removed Pituitary Adenomas

Adenoma type	EGF	EGFr	EGF + EGFr
Somatotroph adenomas, total $n = 10$			
Densely granulated, $n = 5$	3	5	2
Sparsely granulated, $n = 5$	3	2	—
Lactotroph adenomas, total $n = 10$			
Sparsely granulated, $n = 5$	6	2	2
Corticotroph adenomas, total $n = 10$			
Densely granulated, $n = 5$	4	4	3
Sparsely granulated, $n = 5$	4	2	3
Glycoprotein hormone-producing adenomas, total $n = 10$			
Null cell, $n = 4$	—	—	—
Oncocytic, $n = 4$	1	—	—
Gonadotroph differentiation, $n = 2$	—	—	—

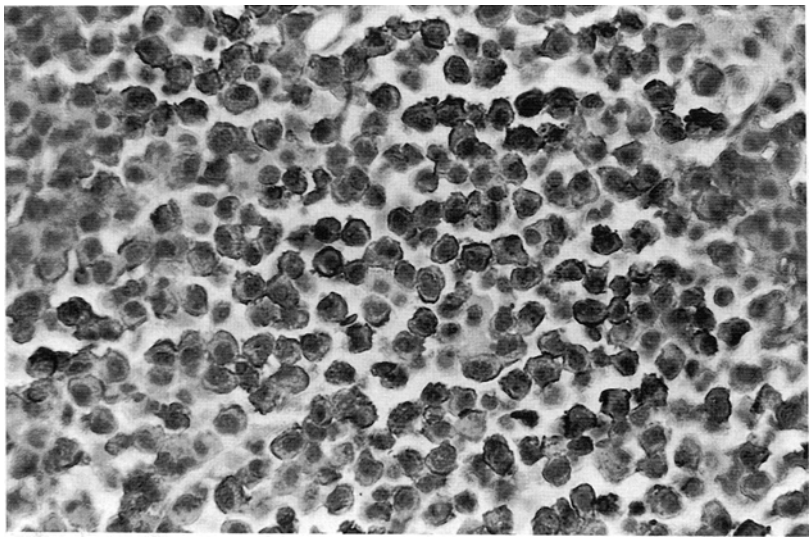

Fig. 5. Strong and diffuse immunoreactivity for EGFr in a corticotroph adenoma. Note variable degree of immunoreactivity among adenoma cells (LSAB, ×460).

types of human cancer, primarily squamous carcinomas and adenocarcinomas, which often overexpress these molecules [24,25]. Regarding endocrine function, there are indications that EGF and EGFr have a role in hormone regulation of several endocrine glands [19,21]. EGF has been reported in GH4C1, GH3/D6, and GH3 rat pituitary tumor cell lines, which synthesize and release GH and PRL [5–8]. EGF also has been found to regulate PRL synthesis and release by GH4C1 pituitary tumor cell line and stimulate TSH secretion in rats [26–28].

Previous studies by Western blotting analysis have shown that human pituitaries contain EGF [13]. They also have demonstrated by ICH the presence of EGF in TSH-, FSH-, and LH-producing cells [11,21] and in the posterior lobe of nontumorous autopsy pituitaries [15]. Recently, EGF was found in >80% of all pituitary adenoma types [22]. In another study, immunoreactivity for EGF was found mostly in nonfunctioning adenomas [21]. In our series, EGF was specifically demonstrated primarily in corticotrophs of nontumorous adenohypophysis. In addition, it was localized in the cytoplasm of 42% of adenomas, comprising 53.5% of functioning tumors, whereas it was negative in the majority of nonfunctioning adenomas. The discrepancy between our findings and studies performed in rats might probably be a result of species differences.

The focal distribution and low incidence of immunoreactivity for EGF in most adenomas and limitation mostly to corticotrophs of nontumorous adenohypophysis, may be attributed to the absence of EGF in some cells or production of small amounts by particular cell types and/or rapid release.

EGF after binding to EGFr, a transforming glycoprotein with intrinsic tyrosine-kinase activity, enhances an array of cellular events that promote cell proliferation and differentiation The receptor-ligand complex is transported to the nucleus and, thus, interacts with DNA to modulate gene transcription [29,30]. EGF binding sites have been found to be present in lactotrophs and somatotrophs of the normal rat pituitary [20,31]. EGFr was reported recently in human nonfunctioning adenomas, whereas

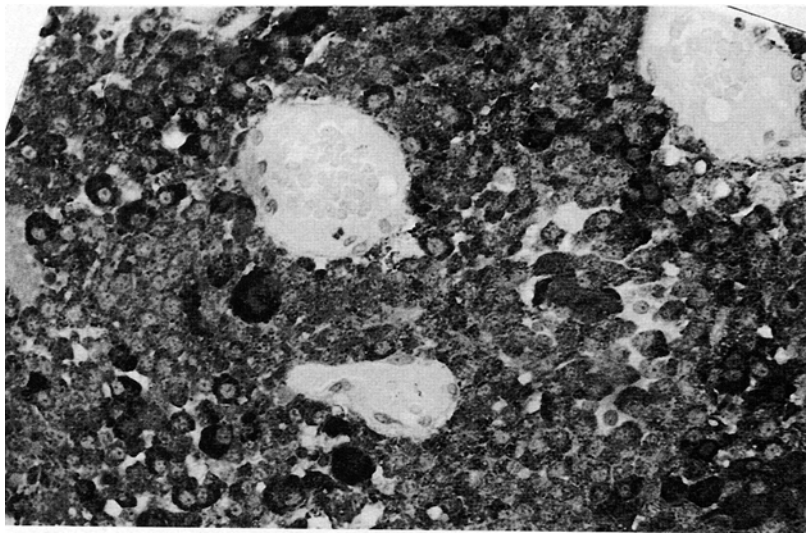

Fig. 6. Peripheral distribution of EGFr immunoreactivity in a sparsely granulated somatotroph adenoma (LSAB, ×460).

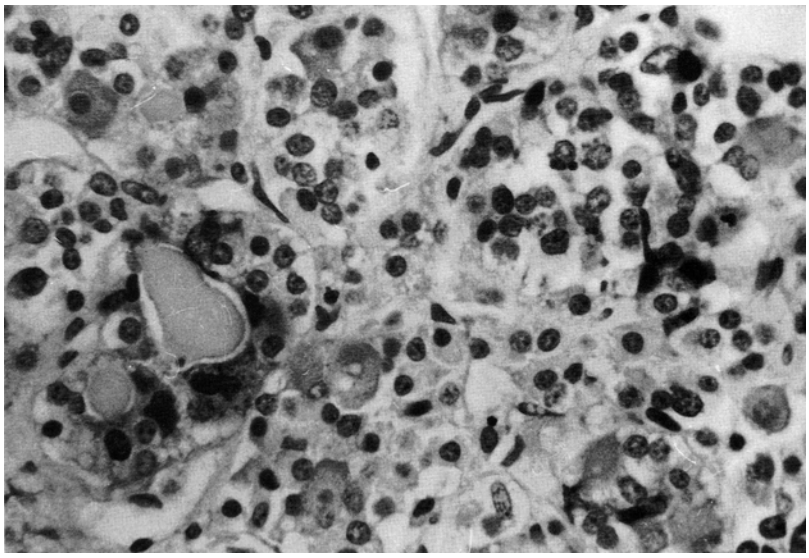

Fig. 7. Selective immunoreactivity for S-100 protein in stellate cells of a nontumorous pituitary gland (LSAB, ×740).

in functioning tumors was found undetectable [21]. In our study, the monoclonal EGFr clone F_4 antibody that specifically recognizes the external epitopes of EGFr was used as in the previous report [32]. EGFr was found in 37.5% of adenomas, representing 50% of functioning tumors. The immunostaining displayed a striking heterogeneity. The distribution was partly peripheral, probably corresponding to cytoplasmic membranes, and intracellular. This pattern could be explained by clonal expansion of EGFr-positive neoplastic cells, during adenoma development and progression. Heterogeneity in EGF binding sites also has been shown by immunoelectron microscopic techniques in rat lactotrophs and somatotrophs [20]. The high frequency and coexpression of EGF and EGFr in corticotroph adenomas suggests their possible particular role in tumor progression and ACTH regulation. Failure to localize EGFr by ICH in some adenoma cells may be attributed to its rapid internalization and subsequent lysosomal degradation; such receptor downregulation occurs after prolonged exposure to peptide hormones or GFs [33].

Regarding the dual intracytoplasmic and membrane distribution of EGFr, it has been suggested that membranous EGFr staining is commonly seen in proliferating cells, whereas intracellular localization is apparent in postmitotic cells [34]. The well-known slow growth of pituitary adenomas may contribute to the lower degree of EGFr expression compared with other neoplasms, such as lung carcinomas [24,25]. In addition, EGFr immunonegativity of nonfunctioning glycoprotein-producing adenomas is correlated with the low proliferation rate and absence or low hormone release by these tumors [23].

In conclusion, this study showed that EGF and EGFr occur relatively frequently in human pituitary adenomas and some nontumorous pituitary cells. Their presence in adenomas may suggest their involvement in cell proliferation and tumor progression. In addition, localization of EGF and EGFr in hormone-producing nontumorous pituitary cells, elements of posterior lobe, and functioning pituitary adenomas raises the questions of whether they play a role in monitoring endocrine activities.

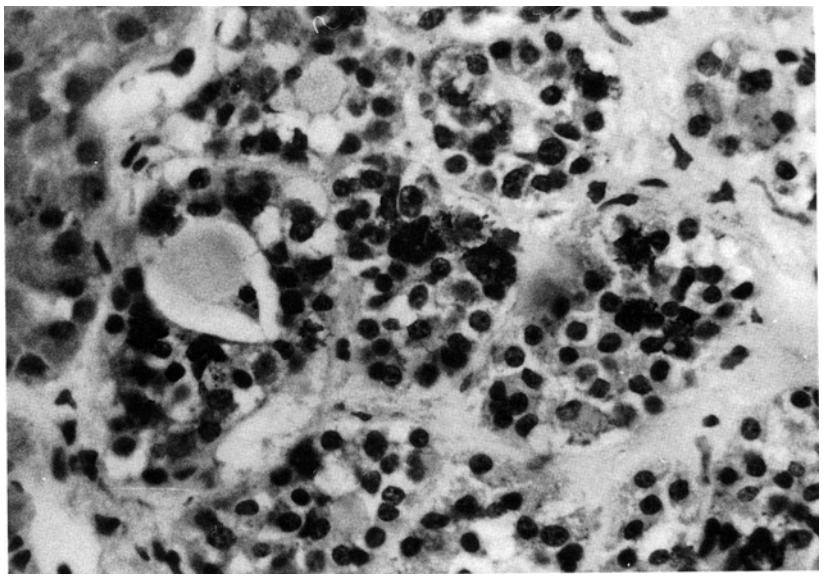

Fig. 8. Selective localization of EGFr in stellate cells (similar to Fig. 7) and in several additional secreting cells (LSAB, ×740).

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