Epidermal growth factor binding sites in human pituitary macroadenomas

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Abstract

The number of epidermal growth factor (EGF) binding sites was determined by competitive binding assays in a series of 46 pituitary macroadenomas. A single concentration of ¹²⁵I-EGF (1 nM) was used for all experiments. In four cases, a displacement curve was obtained by adding increasing concentrations of cold EGF, and Scatchard analysis showed the presence of two classes of EGF binding sites, with $K_{d1}=0.62 \pm 0.23$ nM and $K_{d2}=53.8 \pm 8.2$ nM for the high- and low-affinity binding sites respectively. The distribution of EGF binding sites was studied in 42 cases by a single-point assay, in the presence and in the absence of a 100-fold cold EGF excess. A non-parametric distribution of EGF binding sites was observed (median 10.2 fmol/mg membrane protein, range 0.0–332.0). EGF-receptor positivity, defined as EGF binding ≥ 10.0 fmol/mg protein, was observed in 23 samples (54.8%), especially in prolactinomas (76.5%, $P<0.05$ vs other tumors taken together) and in gonadotrope adenomas (62.5%). EGF binding was higher in invasive than in non-invasive adenomas (median: 12.8 vs 0.0 fmol/mg membrane protein, $P=0.047$), and especially in adenomas

invading the sphenoid sinus (median 26.7 fmol/mg membrane protein, $P=0.008$ vs other adenomas). EGF binding also tended to increase with the grade of supra/extrasellar extension according to Wilson ($P=0.15$). Sex steroid receptors (SSRs) were simultaneously determined in both cytosolic and nuclear fractions of 31 pituitary adenomas. Estrogen and progesterone receptors were determined by an enzyme-linked immunoassay and androgen receptors by a competitive binding assay with [³H]methyltrienolone. No correlation could be found between EGF binding and either the gender and gonadal status of the patients, or the expression of SSRs by the adenomas. We conclude that the EGF family of growth factors may play a role in the evolution of a significant subset of human pituitary adenomas, especially in their invasiveness, and that a high EGF binding capacity may represent an additional marker of aggressiveness for these tumors. Sex steroids do not appear to have a significant role in the regulation of EGF binding *in vivo* in these tumors.

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Introduction

Pituitary tumors are frequent benign neoplasia, accounting for about 10% of symptomatic endocranial neoplasms. In addition, autoptic studies indicate that silent pituitary adenomas – mostly microadenomas – are present in up to 10–20% of the general population (Molitch & Russell 1990). Therefore, attempts to identify factors which can promote or inhibit the clinical expression of these tumors – i.e. the ability to produce pituitary hormones in excess, leading to a specific syndrome of hormone hypersecretion, and the increase in proliferation rate and invasiveness, leading to neurological manifestations – are of special importance for the understanding of the pathogenesis of these tumors, and for the development of new thera-

peutic strategies (von Mehren & Weiner 1996, Huang & Heimbrook 1997). Pituitary tumors are mostly monoclonal in origin (Herman *et al.* 1990), indicating that primary genetic alterations can determine the clonal expansion of abnormal cells. However, few such abnormalities have been identified in these tumors (for review see Shimon & Melmed 1997). On the other hand, pituitary tumor cell function and/or proliferation can be modulated by a variety of extracellular factors, including steroid hormones (Jaffrain-Rea *et al.* 1996), cytokines and growth factors (for review see Shimon & Melmed 1997).

The epidermal growth factor (EGF) family of growth factors is able to modulate the proliferation, secretion and differentiation of most endocrine glands (for review see Fisher & Lakshman 1990). EGF is normally present in the

pituitary, where it appears to be produced by subpopulations of all hormone-producing cell types, and especially by gonadotropes and thyrotropes (Kasselberg *et al.* 1985, Mouihate *et al.* 1996). The epidermal growth factor-receptor (EGF-R) is also normally expressed by anterior pituitary cells (Fan & Childs 1995), with EGF binding sites being localized mainly to lactotrope and somatotrope cells (Chabot *et al.* 1986). The EGF-R has an intrinsic tyrosine kinase activity, and inhibition of tyrosine kinase activities has recently been shown to decrease [^3H]thymidine uptake in primary cultures of human pituitary adenomas (Jones *et al.* 1997). EGF can modulate the secretion of pituitary hormones in both normal (Ikeda *et al.* 1984, Miyake *et al.* 1985, Childs *et al.* 1991) and tumor pituitary cells (Schonbrunn *et al.* 1980, Aanestad *et al.* 1993). Transforming growth factor- α (TGF- α), a member of the EGF family, which can thus also act through the EGF-R on target cells, has been detected in the normal pituitary gland (Kobrin *et al.* 1987, Ezzat *et al.* 1995, Fan & Childs 1995), and has been implicated in pituitary tumorigenesis (Finley & Ramsdell 1994).

There is recent evidence that the EGF-R mRNA is expressed by a significant number of human pituitary adenomas (LeRiche *et al.* 1996), and the expression of the corresponding protein was detected by immunohistochemical studies (Chaidarun *et al.* 1994a, LeRiche *et al.* 1996). Surprisingly, the ability of EGF to bind pituitary adenoma cells has not been documented (Birman *et al.* 1987). In addition, although both EGF and EGF-R can be regulated by sex steroids in hormone-dependent tissues (Mukku & Stancel 1985, Fisher & Lakshmanan 1990, Mouihate & Lestage 1995), no data are available concerning possible relationships between sex steroids and the EGF/EGF-R system in human pituitary tumors. The aim of this study was to investigate further the presence of EGF binding sites in a representative series of pituitary tumors and, if present, to look for possible correlations between EGF binding and the bio-clinical characteristics of the patients and tumors, including the expression of cellular receptors for sex steroids.

Materials and Methods

Patients and tumors

Forty-six patients with pituitary macroadenomas (diameter ≥ 1.0 cm) were operated on for medical reasons by either a transsphenoidal ($n=40$) or a transcranial ($n=6$) approach. There were 26 men (mean age: 45.5 ± 13.3 years) and 20 women (mean age: 47.3 ± 13.6 years). Amenorrhea-galactorrhea was present in 4 women, and typical clinical features of acromegaly in 9 cases. Neurological symptoms were shown by 24 patients, with 19 out of the 24 presenting with visual field defects. Nine patients had recurrent adenomas. Pre-operative RIA measurements of plasma prolactin (PRL), growth hormone (GH), thyroid-

Figure 1 (A) An example of the displacement curve obtained in a case of null cell pituitary macroadenoma by incubating a single concentration of ^{125}I -EGF (1 nM) with increasing concentrations of cold EGF. (B) Analysis of the displacement curve according to Scatchard indicates the presence of two types of EGF binding sites, one with a high affinity (K_{d1} 0.78 nM) and one with a low affinity (K_{d2} 41 nM) binding site.

stimulating hormone (TSH), free thyroxine (FT_4), adrenocorticotropin (ACTH), cortisol, luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone and 17β -estradiol were carried out in all cases using available commercial kits. Hypogonadism in men was defined by plasma testosterone less than 2.5 ng/ml. Tumor volume was evaluated by pre-operative computerized tomodensitometry (TDM) and/or nuclear magnetic resonance imaging (NMR) in all cases. Thirty tumors were defined as invasive according to pre-operative radiological criteria and/or to intra-operative surgical findings, and twelve were non-invasive. The degree of supra/extrasellar extension was defined according to Wilson's criteria (Wilson 1984). Immunohistochemical examination of pituitary tumors was performed on paraffin-embedded tissue sections with polyclonal rabbit antibodies (anti-PRL, anti-GH, anti-FSH, anti-LH, anti-TSH, anti-ACTH;

Figure 2 Distribution of EGF binding sites according to a single-point assay in a series of 42 human pituitary macroadenomas. Median value: 10.2 fmol/mg membrane protein (range 0–332). EGF-R positivity was defined by EGF binding values ≥ 10 fmol/mg protein and is represented by the dotted area.

Orthodiagnostic Systems, Raritan, NJ, USA), using the streptavidin-biotin detection system. Immunohistochemical data were lacking in one case. Tumors were finally classified as: prolactinomas ($n=17$), gonadotrope adenomas ($n=9$), null cell adenomas ($n=8$), pure GH-secreting adenomas ($n=7$), mixed GH/PRL-secreting adenomas ($n=3$), silent ACTH-secreting adenoma ($n=1$), including nine recurrent adenomas consisting of prolactinomas ($n=2$), null cell adenomas ($n=2$), pure GH-secreting adenomas ($n=2$), mixed GH/PRL-secreting adenomas ($n=2$), and gonadotrope adenomas ($n=1$).

Tissue preparation

Frozen tumor samples were homogenized in 2 ml TED buffer (0.01 M Tris-HCl, 0.001 M EDTA, 0.001 M dithiothreitol, 0.01 M sodium molybdate, 10% glycerol, pH 7.4 at 25 °C) and subsequently centrifuged at 800 g (ALC 4227 R refrigerated centrifuge) for 10 min to obtain cytoplasmic and nuclear fractions. Cytosolic fractions and nuclear extracts were obtained as previously described (Jaffrain-Rea *et al.* 1996). The cytoplasmic fraction was then ultracentrifuged at 30 000 g for 45 min to obtain the membrane pellet, which was resuspended in 2.5 ml 0.5 M $MgCl_2$ and incubated for 2 min at 25 °C for dissociating endogenous ligand from EGF-R. After the addition of 7.5 ml cold TE buffer (0.01 M Tris-HCl, 0.001 M EDTA, pH 7.4 at 25 °C), the membranes were ultracentrifuged at 30 000 g for 30 min. The final pellets were then

re-suspended in TE buffer (final protein concentration 0.1 to 0.7 mg/ml). Protein concentration was determined according to Bradford (1976).

EGF binding experiments

All EGF binding experiments were carried out in triplicate with a single 1 nM concentration of ^{125}I -EGF (specific activity 150–200 mCi/mg, New England Nuclear, Dupont de Nemours, France), according to a modified published method (Lubrano *et al.* 1993). In 4 cases, including 2 null cell, 1 prolactinoma, and 1 pure GH-secreting adenoma, increasing concentrations of cold EGF (0.0156–15 nM) were added to 1 nM ^{125}I -EGF in order to obtain a displacement curve, and the non-linear Scatchard plots were analyzed on a personal computer using the LIGAND software according to the two-site model (Munson & Rodbard 1980). In most cases, a single-point saturation assay was obtained by determining EGF binding in the presence or absence of a 100-fold cold EGF concentration. In all cases, the incubation was performed at 25 °C for 60 min and, at the end of the incubation period, the EGF/EGF-R complexes were precipitated by polyethylene glycol. The radioactivity was counted in a Packard gamma-counter with an efficiency ratio of 48%. In agreement with most studies based on radioligand assays for EGF-R determination (for review see Klijn *et al.* 1992), EGF-R positivity was defined by EGF binding values ≥ 10 fmol/mg protein.

Table 1 Distribution of EGF binding sites in a series of 42 human pituitary macroadenomas according to patients' and tumors' characteristics, except hormone cell type

	<i>n</i>	Median (fmol/mg protein)	Range (fmol/mg protein)	Positivity
Gender/gonadal status				
Men	23	12.3	0–233	56.5%
<i>Hypogonadal</i>	11	10.0	0–148	6/11
<i>Eugonadal</i>	9	12.3	0–233	5/9
Women	19	10.3	0–332	52.6%
<i>Amenorrhea/menopause</i>	14	9.0	0–332	7/14
<i>Eugonadal</i>	5	10.3	0–123	3/5
Tumor recurrence				
No	33	12.0	0–332	57.5%
Yes	9	5.0	0–101	44.4%
Tumor volume				
Intrasellar	9	4.8	0–21.9	22.2%
Extrasellar	33	12.5	0–332	60.6%
Tumor invasivity				
Non-invasive	12	0.0	0–71.2	41.7%
Invasive	30	12.8 ^a	0–332	63.3%

^a*P*=0.047 vs non-invasive adenomas.

Cellular receptors for sex steroids

Cytosolic receptors for estrogens (ERs), progesterone (PgRs) and androgens (ARs) were studied in all cases, and the corresponding nuclear receptors were studied in 31 cases, according to previously published methods (Jaffrain-Rea *et al.* 1996). In brief, ERs and PgRs were determined by an enzyme-linked immunoassay using commercially available specific monoclonal antibodies (Abbott, Chicago, IL, USA), and ARs were determined by competitive binding with [³H]methyltrienolone (R1881), in the presence of triamcinolone acetonide to avoid significant binding of methyltrienolone to the PgR (Ekman *et al.* 1982). Positivity for sex steroid receptors (SSRs) was defined by cytosolic concentrations ≥ 3 fmol/mg protein and/or nuclear concentrations ≥ 20 fmol/mg DNA.

Statistical analysis

Unless otherwise specified, results are expressed as median values and range. Only EGF binding values obtained by the single-point saturation assay were considered for statistical comparison of subgroups. Distribution of positive cases for EGF binding were compared by the Chi-square test, adequately modified by Yates correction if necessary. Because of the non-parametric distribution of EGF-binding values, bivariate analysis was performed by the Mann-Whitney U-test and multivariate analysis by the Kruskal-Wallis test. Correlations between the concentration of EGF binding sites and the concentrations of SSRs were studied by both linear regression and the

non-parametric Spearman correlation test. Statistical analysis was supported by the Statview 4.0 software for MacIntosh.

Results

Scatchard analysis of EGF binding

Figure 1 shows the non-linear displacement curve of ¹²⁵I-EGF obtained in a sample of null cell pituitary tumor (panel A) and its resolution into a two-sites binding model according to Scatchard analysis (panel B). Similar results were obtained in all cases studied, indicating the presence of both high- and low-affinity binding sites in these tumors, with a *K*_{d1}=0.62 ± 0.23 nM for the high affinity site and a *K*_{d2}=53.8 ± 8.2 nM for the low affinity site (means ± s.d.). Similar results were also obtained in positive control samples from human benign prostatic hyperplasia (data not shown).

EGF binding according to the single-point assay

Analysis of 42 pituitary macroadenomas revealed a highly variable, non-parametric distribution of EGF binding sites, ranging from 0 to 332.0 fmol/mg protein, with a median value of 10.2 fmol/mg protein (Fig. 2). EGF-R positivity (≥ 10 fmol/mg protein) was present in 23 cases (54.8%). Patients' gender and gonadal status were found to have no influence on EGF binding (Table 1).

Distribution of EGF binding sites according to recurrence, tumor volume and invasivity

As shown in Table 1, no statistical difference was found between recurrent and non-recurrent tumors. In contrast, EGF binding was higher in invasive adenomas than in non-invasive adenomas ($P=0.047$) and, accordingly, EGF-R positivity tended to be higher in the former group (63.3% vs 33.3%, $\chi^2=3.11$, $P<0.1$). In particular, positivity for EGF-R was present in 90% of the tumors invading the sphenoidal sinus ($P<0.02$ vs other tumors) and EGF binding was significantly higher in this group than in other tumors (median 26.7 vs 4.9 fmol/mg protein, $P=0.009$) (Fig. 3, panel A).

EGF binding also tended to increase with the degree of supra/extrasellar extension ($P=0.15$) (Fig. 3, panel B). High values of EGF binding (≥ 30 fmol/mg protein) were significantly more frequent in C-D-E than in O-A-B adenomas defined according to Wilson (1984) (38.9% vs 8.8%, $\chi^2=3.94$, $P<0.05$).

Distribution of EGF binding sites in hormone-producing cell types

EGF binding sites were mainly detected in prolactinomas (EGF-R positivity 13/17=76.5%) and in gonadotrope pituitary adenomas (EGF-R positivity 5/8=62.5%) (Table 2). Overall, EGF-R positivity was more frequent in prolactinomas than in non-prolactinoma tumors ($\chi^2=5.43$, $P<0.05$) and EGF binding values tended to be higher in the former group (median 21.9 vs 5.0 fmol/mg protein, $P=0.06$). When only non-invasive tumors were considered, EGF positivity was observed in 2/3 gonadotrope adenomas, 1/3 prolactinomas, 1/3 pure GH-secreting adenomas, but not in null cell adenomas (0/4).

Patients' gender and dopamine-agonist therapy had no influence on EGF binding in the prolactinoma group (data not shown).

Correlation between EGF binding and expression of SSRs in pituitary tumors

The distribution of EGF binding in pituitary adenomas according to expression of SSRs is summarized in Table 3. No correlation was found between EGF binding values and either the cytosolic or nuclear concentrations of ERs, PgRs and ARs ($r<0.15$ in all cases). There were also no positive or negative associations between cytosolic, nuclear or total ERs, PgRs and ARs and EGF-R positivity.

Discussion

To our knowledge, this is the first report showing the presence of EGF binding sites in a series of human pituitary macroadenomas. The presence of EGF binding

Figure 3 (A) Distribution of EGF binding sites in pituitary macroadenomas invading or not the sphenoid sinus. Median values are indicated for each group, and were significantly higher in adenomas invading the sphenoid sinus than in other adenomas, as indicated by ** ($P<0.02$). EGF-R positivity was significantly more frequent in adenomas invading the sphenoid sinus than in other adenomas (90.0% vs 43.7%, $P<0.02$). (B) Distribution of EGF binding sites in pituitary adenomas classified according to Wilson's criteria. EGF binding tended to increase with the grade of supra/laterosellar extension ($P=0.15$). High EGF binding values (≥ 30 fmol/mg protein) were more frequent in C-D-E than in O-A-B adenomas (38.9% vs 8.8%, $P<0.05$).

sites is consistent with the presence of EGF-R mRNA and EGF-R protein detected by immunohistochemistry in a significant subset of these tumors (Chaidarun *et al.* 1994a, LeRiche *et al.* 1996). The reasons why EGF-binding was previously detected in the normal human pituitary gland but not in human pituitary tumors are unclear (Birman *et al.* 1987). In this study, two classes of EGF binding sites were shown by Scatchard analysis, with respective high and low affinities, which may reflect the capacity of EGF-R to present allosteric modifications—especially dimerization—following ligand binding (King & Cuatrecasas 1982, Boni-Schnetzler & Pilch 1987). This is

Table 2 Distribution of EGF binding sites in a series of 41 human pituitary macroadenomas according to hormone cell type

	<i>n</i>	Invasivity	Median (fmol/mg protein)	Range (fmol/mg protein)	Positivity
Tumor					
FSH-secreting	8	5/8	20·6	0–332	5/8
GH/PRL-secreting	3	3/3	6·5	0–101	2/3
GH-secreting	6	3/6	5·0	0–59	1/6
Null cell	6	2/6	0	0–65	1/5
ACTH	1	1/1	19·9	—	1/1
All non-PRL	24	58·3%	3·0	0–332	41·7%
PRL-secreting	17	82·3%	21·9 ^a	0–233	76·5% ^b

^a*P*=0·063 vs non-prolactinoma tumors (non-significant trend); ^b*P*<0·05 vs non-prolactinoma tumors.

in agreement with previous reports on human tumors of various origins (Nicholson *et al.* 1988, Lubrano *et al.* 1993), although other studies have reported the presence of a single class of high affinity binding sites (Yoshida *et al.* 1993), depending on experimental conditions and on the concentration of EGF binding sites themselves (Bolufer *et al.* 1990). The determination of the binding capacity for the high affinity binding site appears to be poorly influenced by the choice of the radioligand assay, and simplified methods, such as two-point assays (Nicholson *et al.* 1988) or single-point saturation assays (Etienne *et al.* 1990, Petrangeli *et al.* 1995), have been developed to allow easier evaluation of EGF-R status for clinical purposes. Because of the limited amount of material available in most pituitary tumors – microadenomas had to be excluded from this study because the concentrations of total membrane protein were too low to be suitable for the radioligand assay – a single-point assay was chosen for the study of EGF-R distribution.

EGF binding sites in the normal pituitary are usually detected in somatotropes and lactotropes (Chabot *et al.* 1986), but the expression of EGF-R by pituitary adenomas is less defined. EGF-R protein immunoreactivity was found to be almost limited to non-functioning adenomas, but gonadotrope adenomas and prolactinomas were poorly represented in these series (Chaidarun *et al.* 1994a). In contrast, using both immunohistochemical methods and reverse transcriptase (RT)-PCR analysis, a variable expression of EGF-R and its mRNA has been reported in all types of secreting and non-secreting tumors, with an overexpression in recurrent somatotrope adenomas (LeRiche *et al.* 1996). Data from the present study further argue for an ubiquitous, although highly variable, expression of the EGF-R in pituitary adenomas. It is difficult to determine whether the high levels of EGF binding detected in prolactinomas reflect an intrinsic characteristic of these tumors, since this result could be partially biased by the high percentage of invasive tumors in this subgroup.

Table 3 Distribution of EGF binding sites in a series of 31 human pituitary macroadenomas according to the expression of sex steroid receptors (SSRs). ERs and PgRs were determined by an enzyme-linked immunoassay (ELISA) with monoclonal antibodies (Abbott), and ARs were determined by a binding assay with [³H]R1881. Both cytosolic and nuclear receptors were determined in these cases, and SSRs positivity was defined by either cytosolic concentrations ≥ 3 fmol/mg protein or nuclear concentrations ≥ 20 fmol/mg DNA respectively. The number of EGF-R positive samples is indicated within parentheses for each subgroup.

Tumor	<i>n</i>	EGF-R positivity (≥ 10 fmol/mg protein)	EGF binding (fmol/mg protein)	
			Median	Range
ER positive	21	61·9% (13)	13·1	0–233
ER negative	10	60·0% (6)	25·0	0–332
PgR positive	15	53·3% (8)	10·0	0–233
PgR negative	16	68·7% (11)	16·2	0–332
AR positive	25	60·0% (15)	16·2	0–233
AR negative	6	66·7% (4)	35·5	0–332

In fact, EGF binding was found to be significantly higher in invasive than in non-invasive pituitary adenomas, especially in those invading the sphenoid sinus, and tended to increase with tumor volume. High values of EGF binding (≥ 30 fmol/mg protein) were observed mainly in tumors with a huge supra/laterosellar extension (C-D-E grading according to Wilson 1984), contrasting with the low levels (≤ 22 fmol/mg protein) observed in intrasellar adenomas. Thus, a high EGF-R expression appears to represent a marker of aggressiveness in a significant subset of pituitary tumors, as reported in a variety of human neoplasia (Smith *et al.* 1989, Toi *et al.* 1990, Lund-Johansen *et al.* 1990, Yoshida *et al.* 1993) and increased EGF-R expression is likely to be a late event in pituitary tumorigenesis.

The mechanisms by which the EGF family of growth factors may promote the proliferation and the invasiveness of pituitary adenoma cells are unclear, since they have multiple and variable effects on pituitary cells, depending on the hormone cell type and on the malignancy of the cells. For instance, EGF inhibits the proliferation of GH/PRL secreting cell strains while inducing their differentiation into lactotropes (Schonbrunn *et al.* 1980, Felix *et al.* 1995). On the other hand, EGF stimulates [3 H]thymidine incorporation by sheep pituitary cells while increasing the percentage of undifferentiated cells (Chaidarun *et al.* 1994b) and enhances the proliferation of human non-functioning pituitary tumor cells *in vitro* (Chaidarun *et al.* 1994a). EGF also stimulates both hormone secretion and cell proliferation in pituitary corticotropes (Childs *et al.* 1991, 1995). EGF and EGF-R were found to be co-expressed in non-functioning pituitary adenomas (Chaidarun *et al.* 1994a), suggesting that autocrine mechanisms involving the EGF/EGF-R system are present in this subgroup. TGF- α immunoreactivity has also been detected in most pituitary adenomas, independent of their cell type (Ezzat *et al.* 1995), and may be especially important for the promotion of PRL/GH-secreting tumors (Finley & Ramsdell 1994). Taken together, these data indicate that the EGF/TGF- α system may be of special importance in the pathogenesis of human pituitary tumors.

Significant correlations have been made between the expression of EGF-R and SSRs in various tumors. In breast cancer, a negative correlation exists between ER/PgR and EGF-R expression (Klijn *et al.* 1992), and ER and EGF-R are considered as positive and negative prognostic markers respectively. In this study, EGF binding was found to be independent of SSRs expression in pituitary adenomas. In particular, EGF binding was similar in ER-positive and ER-negative tumors. Thus, although the expression of both ER and EGF-R tends to increase with tumor volume and invasiveness in pituitary adenomas (Jaffrain-Rea *et al.* 1996), the overexpression of ER and EGF-R appears as independent prognostic markers. On the other hand, both EGF secretion and EGF-R expres-

sion are regulated by various factors including sex steroids (for review see Carpenter & Wahl 1990). EGF binding is generally higher in male than in female tissues, including the normal pituitary gland (Birman *et al.* 1987), reflecting EGF-R induction by androgens. The expression of both SSRs (McGinnis *et al.* 1981, Handa *et al.* 1986) and EGF-R (Armstrong & Childs 1997a) also vary throughout the estrous cycle, reflecting cyclic variations in estradiol concentrations (Armstrong & Childs 1997b). In contrast to previous data from our laboratory, which strongly supported the influence of the gonadal environment on SSRs expression by human pituitary adenomas (Jaffrain-Rea *et al.* 1996), patients' gender and gonadal status at the time of surgery had no significant influence on EGF binding in this series. Because of the reduced number of subjects included in each subgroup, these results do not rule out the presence of regulating effects of sex steroids on EGF-R expression in these tumors, but suggest that such effects, if present, are not of major physiopathological importance *in vivo*.

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References

- Aanestad M, Rotnes JS, Torjesen PA, Haug E, Sand O & Bjoro T 1993 Epidermal growth factor stimulates the prolactin synthesis and secretion in rat pituitary cells in culture (GH₄C₁ cells) by increasing the intracellular concentration of free calcium. *Acta Endocrinologica* **128** 361–366.
- Armstrong JL & Childs GV 1997a Changes in expression of epidermal growth factor receptors by anterior pituitary cells during the estrous cycle: cyclic expression by gonadotropes. *Endocrinology* **138** 1903–1908.
- Armstrong JL & Childs GV 1997b Regulation of expression of epidermal growth factor receptors by epidermal growth factor and estradiol: studies in cycling female rats. *Endocrinology* **138** 5434–5441.
- Birman P, Michard M, Li JV, Peillon F & Bression D 1987 Epidermal growth factor binding sites, present in the normal human and rat pituitaries, are absent in human pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* **65** 275–281.
- Bolufé P, Miralles F, Rodriguez A, Vasquez C, Lluch A, Garcia-Conde J & Olmos T 1990 Epidermal growth factor receptor in human breast cancer: correlation with cytosolic and nuclear ER receptors and with biological and histological tumor characteristics. *European Journal of Cancer* **26** 283–290.
- Boni-Schnetzler M & Pilch PF 1987 Mechanism of epidermal growth factor receptor autophosphorylation and high affinity binding. *Proceedings of the National Academy of Sciences of the USA* **84** 7832–7836.
- Bradford MM 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Analytical Biochemistry* **72** 248–259.
- Carpenter G & Wahl MI 1990 The epidermal growth factor family. *Handbook of Experimental Pharmacology* **951** 69–171.

- Chabot JG, Walker P & Pelletier G 1986 Distribution of epidermal growth factor binding sites in the rat adult pituitary gland. *Peptides* **7** 45–50.
- Chaidarun SS, Eggo M, Sheppard MC & Stewart PM 1994a Expression of epidermal growth factor (EGF), its receptor and related oncoprotein (*erbB-2*) in human pituitary tumors and response to EGF *in vitro*. *Endocrinology* **135** 2012–2021.
- Chaidarun SS, Eggo MC, Stewart PM, Barber PC & Sheppard MC 1994b Role of growth factors and estrogens as modulators of growth, differentiation and expression of gonadotropin subunit genes in primary cultured sheep pituitary cells. *Endocrinology* **134** 935–944.
- Childs GV, Patterson J, Unabia G, Rougeau D & Wu P 1991 Epidermal growth factor enhances ACTH secretion and expression of POMC mRNA by corticotropes in mixed and enriched cultures. *Molecular and Cellular Neuroscience* **2** 235–243.
- Childs GV, Rougeau D & Unabia G 1995 Corticotropin releasing hormone and epidermal growth factor: mitogens for anterior pituitary corticotropes. *Endocrinology* **136** 1595–1602.
- Ekman P, Barrack ER & Walsh PC 1982 Simultaneous measurement of progesterone and androgen receptors in human prostate: a microassay. *Journal of Clinical Endocrinology and Metabolism* **55** 1089–1099.
- Etienne MC, Formento JL, Francoual M, Formento P, Fischel JL, Namer M, Frenay M & Milano G 1990 Epidermal growth factor receptor assay: validation of a single point saturation method in breast cancer. *European Journal of Cancer* **26** 181 (Abstract 139).
- Ezzat S, Walpole IA, Ramyar L, Smyth HS & Asa SL 1995 Membrane-anchored transforming growth factor- α in human pituitary adenoma cells. *Journal of Clinical Endocrinology and Metabolism* **80** 534–539.
- Fan X & Childs GV 1995 Epidermal growth factor and transforming growth factor- α messenger ribonucleic acids and their receptors in the rat anterior pituitary: localization and regulation. *Endocrinology* **136** 2284–2293.
- Felix R, Meza U & Cota G 1995 Induction of classical lactotropes by epidermal growth factor in rat pituitary cell culture. *Endocrinology* **136** 939–946.
- Finley EL & Ramsdell JS 1994 A transforming growth factor- α pathway is expressed in GH4C1 rat pituitary tumors and appears necessary to tumor formation. *Endocrinology* **135** 416–422.
- Fisher DA & Lakshmanan J 1990 Metabolism and effects of epidermal growth factor and related growth factors in mammals. *Endocrine Reviews* **11** 418–442.
- Handa RJ, Reid DL & Resko JA 1986 Androgen receptors in brain and pituitary of female rats: cyclic changes and comparisons with the male. *Biology of Reproduction* **34** 293–303.
- Herman V, Fagin J, Gonsky R, Kovacs K & Melmed S 1990 Clonal origin of pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* **71** 1427–1433.
- Huang PS & Heimbrook DC 1997 Oncogene products as therapeutic targets for cancer. *Current Opinion in Oncology* **9** 94–100.
- Ikeda H, Mitsuhashi T, Kubota K, Kuzuya N & Uchimura H 1984 Epidermal growth factor stimulates growth hormone secretion from superfused rat adenohypophyseal fragments. *Endocrinology* **115** 556–558.
- Jaffrain-Rea ML, Petrangeli E, Oortolani F, Fraioli B, Lise A, Esposito V, Spagnoli LG, Tamburrano G, Frati L & Gulino A 1996 Cellular receptors for sex steroids in human pituitary adenomas. *Journal of Endocrinology* **151** 175–184.
- Jones TH, Justice SK & Price A 1997 Suppression of tyrosine kinase activity inhibits [3 H]thymidine uptake in cultured human pituitary tumor cells. *Journal of Clinical Endocrinology and Metabolism* **82** 2143–2147.
- Kasselberg AG, Orth DN, Gray ME & Stahlman MT 1985 Immunocytochemical localization of human epidermal growth factor/urogastrone in several human tissues. *Journal of Histochemistry and Cytochemistry* **33** 315–322.
- King AC & Cuatrecasas 1982 Resolution of high and low affinity epidermal growth factor receptors: inhibition of high affinity component by low temperature, cycloheximide and phorbol esters. *Journal of Biological Chemistry* **257** 3053–3060.
- Klijn JGM, Berns PMJJ, Schmitz PIM & Foekens JA 1992 The clinical significance of epidermal growth factor receptor (EGF-R) in human breast cancer: a review on 5232 patients. *Endocrine Reviews* **13** 3–17.
- Kobrin MS, Asa SL, Samsundar J & Kudlow JE 1987 Alpha-transforming growth factor in bovine anterior pituitary gland: secretion by dispersed cells and immunohistochemical localization. *Endocrinology* **121** 1412–1416.
- LeRiche VK, Asa SL & Ezzat S 1996 Epidermal growth factor and its receptor (EGF-R) in pituitary adenomas: EGF-R correlates with tumor aggressiveness. *Journal of Clinical Endocrinology and Metabolism* **81** 656–662.
- Lubrano C, Toscano V, Petrangeli E, Spera G, Trotta MC, Rombola N, Frati L, Di Silverio F & Sciarra F 1993 Relationship between epidermal growth factor and its receptor in human benign prostatic hyperplasia. *Journal of Steroid Biochemistry and Molecular Biology* **46** 463–468.
- Lund-Johansen M, Bjerkvig R, Humphrey PA, Bigner SH, Bigner DD & Laerum OD 1990 Effect of epidermal growth factor on glioma cell growth, migration and invasion *in vitro*. *Cancer Research* **50** 6039–6044.
- McGinnis MY, Krey LC, McLusky NJ & McEwen BS 1981 Steroid receptor levels in intact and ovariectomized estrogen-treated rats: an examination of quantitative, temporal and endocrine factors influencing the efficacy of an estradiol stimulus. *Neuroendocrinology* **33** 158–165.
- von Mehren M & Weiner LM 1996 Monoclonal antibody-based therapy. *Current Opinion in Oncology* **8** 493–498.
- Miyake A, Tasaka K, Otsuka S, Kohmura H, Wakimoto H & Aono T 1985 Epidermal growth factor stimulates secretion of rat pituitary luteinizing hormone *in vitro*. *Acta Endocrinologica* **108** 175–178.
- Molitch ME & Russell EJ 1990 The pituitary ‘incidentaloma’. *Annals of Internal Medicine* **112** 925–931.
- Mouihate A & Lestage J 1995 Estrogen increases the release of epidermal growth factor from individual pituitary cells in female rats. *Journal of Endocrinology* **146** 495–500.
- Mouihate A, Verrier D & Lestage J 1996 Identification of epidermal growth factor-secreting cells in the anterior pituitary of lactating female rats. *Journal of Endocrinology* **148** 319–324.
- Mukku VR & Stancel GM 1985 Regulation of epidermal growth factor receptor by estrogen. *Journal of Biological Chemistry* **260** 9820–9824.
- Munson PJ & Rodbard D 1980 LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Analytical Biochemistry* **107** 220–239.
- Nicholson S, Sainsbury JRC, Needham GK, Chambers JR, Farndon JR & Harris AL 1988 Quantitative assays of epidermal growth factor receptor in human breast cancer: cut-off points of clinical relevance. *International Journal of Cancer* **42** 36–41.
- Petrangeli E, Lubrano C, Ravenna L, Vacca A, Cardillo MR, Salvatori L, Sciarra F, Frati L & Gulino A 1995 Gene methylation of estrogen and epidermal growth factor receptors in neoplastic and perineoplastic breast tissues. *British Journal of Cancer* **72** 973–975.
- Schonbrunn A, Krasnoff M, Westendorf JM & Tashjian Jr AH 1980 Epidermal growth factor and thyrotropin releasing hormone act similarly on a clonal pituitary cell strain: modulation of hormone production and inhibition of cell proliferation. *Journal of Cell Biology* **85** 786–797.
- Shimon I & Melmed S 1997 Pituitary tumor pathogenesis. *Journal of Clinical Endocrinology and Metabolism* **82** 1675–1681.

- Smith K, Fennely JA, Neal DE, Hall RR & Harris AL 1989 Characterization and quantitation of epidermal growth factor receptor in invasive and superficial bladder tumors. *Cancer Research* **49** 5810–5815.
- Toi M, Nakamura T, Mukaida H, Wada T, Osaki A, Yamada H, Toge T, Niimoto M & Hattori T 1990 Relationship between epidermal growth factor receptor status and various prognostic factors in human breast cancer. *Cancer* **65** 1980–1984.
- Wilson CB 1984 A decade of pituitary microsurgery. *Journal of Neurosurgery* **61** 814–833.
- Yoshida K, Tosaka A, Takeuchi S & Kobayashi 1993 Epidermal growth factor receptor content in human renal cell carcinomas. *Cancer* **73** 1913–1918.

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