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Epidermal growth factor receptor in adenocarcinoma of the kidney

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Summary. Epidermal growth factor receptor (EGF-R) was estimated in Hypernephroma by saturation analysis using 125-I EGF as ligand. Tissue levels of this oncogene related protein were increased five-fold (24 to 99 fmol/mg protein) in comparison with the surrounding tumor free tissue (3 to 18 fmoles/mg protein). There was no apparent correlation to the stage of tumor growth or tumor differentiation and there was no correlation of EGF serum levels with tumor growth. EGF serum levels in the tumor patients did not exceed levels in control patients.

Key words: Epidermal growth factor receptor – Kidney carcinoma

Introduction

Reports on epidermal growth factor (EGF)/beta-urogastrone involvement in malignant growth of the bladder [1, 2], the lung [2, 4, 17] mammary carcinoma [5, 7, 10, 14] and in prostate carcinoma [9, 8, 15] indicate an important role of this peptide in the development of normal and malignant tissue.

Epidermal growth factor (EGF) is an ubiquitous 6,000 MW protein with several possible locations of synthesis [6]; platelets are probably the most important source [11]. Reports by Hirata and Orth show EGF in considerable amounts in other tissues [6]. For example in kidney tissue EGF was present at 5.5 ng/mg wet weight, one of the highest levels for all tissues tested.

EGF is bound to a receptor protein of 175,000 MW on the cell membrane (EGF-R) which belongs to the family of receptor tyrosine kinases (RTK) [20]. It is important to note a close relationship between the RTK and the oncogene c-erbB.Interest in the role of EGF-R in malignant growth was initiated by the observation that the v-erb-B oncogene of avian erythroblastosis virus is derived from the chicken EGF-receptor. More recently, it was established that another oncogene c-erbB2/neu was increased in mammary carcinomas and a strong correlation between poor prognosis and the amplification of this oncogene was established [16]. Kidney tumors appear to have been neglected as possible targets of observation with respect to oncogenes and EGF-receptors.

As little is known about the factors influencing the growth of adenocarcinomas of the kidney and as reports on the response of hypernephroma to steroid hormone therapy are somewhat controversial [13], we initiated studies of new aspects of diagnosis and treatment of this carcinoma.

Malignant growth in the kidney occurs at a frequency of 1% of all types of cancer and 75% are adenocarcinomas i.e. epithelial tumors of the kidney of parenchymous origin. The neoplasms present themselves as encapsulated growths with occasional necrosis within the mostly durable tissue. Tumor cells are of either clear, granular or anaplastic appearance.

Since the growth of hypernephromas mostly go unnoticed by the patient it is detected only at very late stages of tumor growth. At the point of detection the tumor often has metastasised. Detection of the tumor by means of tumor markers hitherto is not possible and occurs mainly by intravenous pyelogram, ultrasound, computer tomography or clinically, by macrohematuria. X-ray is used for follow up of the tumor to detect metastasis to the lung in its early stages. Metastatic growth is treated either by surgery or by symptomatic treatment.

Material and methods

Patients

Thirteen patients (eight females and five males) undergoing surgery for removal of tumors in the kidney were included. The average age

Table 1. See text

Case Nr.	EGF-Receptor	EGF-Receptor	EGF (Serum)	EGF (Cytosol)	EGF (Cytosol)	TNF (Serum)	Staging	Tumor
	Tumor fmoles/mg protein	Normal tissue fmoles/mg protein	nmoles/l	Tumor pmoles/mg protein	Normal tissue pmoles/mg protein	pg/ml		
1	24	7	0.184	nd	nd	0	pT2NoMoG2	Hypernephroma
2	51	8	0.3	9.6	23.9	0	pT2NoMoG2	Hypernephroma
3	4	nd	nd	nd	nd	nd	pT2N2M1G2	Hypernephroma
4	54 9	6	0.033	0	0	40	pT2NxM1G1	Hypernephroma Necrosis
5	28	5	0.117	nd	nd	0	pT2NxMxG2	Hypernephroma
6	5	3	0.03	0	0	84	pT3NoMoG1	Urothelial-Ca
7	32	3	nd	0	7.7	nd	pT3NoMoG1	Hypernephroma
8	31	10	nd	0	0	26	pT3NoMoG2	Hypernephroma
9	36	14	0.434	0	0	0	pT3NoMxG2–3	Hypernephroma
10	66	5	0.134	0	0	0	pT3NxMxG2	Hypernephroma
11	22	5	nd	0	9.2	nd	pT3NxMxG2	Hypernephroma
12	99	18	0.15	0	nd	0	pT3NxMxG2	Hypernephroma
13	4	3	0.084	6.7	13.5	<12.8		Angio lipo leioma

nd = not determined

was 58.7 years (range 28–79 years). Tissue samples were examined macroscopically and sent to the laboratory for EGF-receptor analysis. The rest of the material was examined histologically by the Pathology department of the hospital (Prof. Dr. Mihatsch).

Before surgery EDTA-plasma was drawn and sent to the laboratory for EGF and TNF (tumor necrosis factor) content. EDTA plasma was collected from a series of post surgery (up to 3 months) patients from outside the hospital for estimation of EGF and TNF.

EGF-receptor-assay

Epidermal growth factor receptor was determined in tumor tissue and tissue apparently tumor free from an adjacent region to the tumor. The determination of EGF-R content was carried out as described by Wyss et al. [19] using the single point assay. In brief: 105,000 G pellet of the kidney carcinoma or the tumor free tissue was dissolved in 20 mmolar HEPES buffer (pH 7.4) containing 10 µg/ml Leupeptin, 2 µg/ml Aprotinin and 10% Glycerol and 0.1% essentially fatty acid-free bovine serum albumin (BSA-FAF) and incubated with 3.6 nM 125-I-EGF (Amersham, 30 µCi/µg). Unspecific binding was determined running parallel samples with 100 fold excess of unlabelled EGF (Sigma). Incubation was carried out at 25°C and stopped after 2 hours by adding 2 ml ice cold washbuffer (20 mM HEPES, pH 7.4) and filtering of the sample through a Durapore filter (Millipore: HVLP, 0.45 µm).

After 2 rinses with buffer the filters were transferred to counting vials containing Instagel (Packard) and counted for radioactivity. Subtraction of unspecific binding from total binding yielded specific binding using the Student's t-test at a level of $P < 0.05$.

Epidermal growth factor (EGF)-radioimmunoassay

The human EGF Radioimmunoassay using a rabbit anti-human EGF from Biomedical Technologies Inc (Stoughton, MA, USA) was used to measure h-EGF in EDTA-Plasma and Cytosol (prepared for androgen receptor assay). Assay sensitivity was 0.04 nmol/l.

Tumor necrosis factor (TNF) assay

Microtiter plates were coated with (5 µg/ml) monoclonal anti-r-h-TNF overnight and incubated, after saturation with BSA-containing phosphate buffer at pH 6 with 50 µl EDTA-plasma or cytosol and incubated overnight in the presence of POD-activated anti-TNF. (The TNF-Assay was a gift from Dr. Gallati, Hoffmann-LaRoche AG, Basel.) Assay sensitivity was 6 pg/ml.

Results

EGF-receptor

Table 1 summarizes the data obtained from 13 patients presenting with kidney tumors. Eleven were histologically typed as clear cell carcinoma of the kidney or hypernephroma. 9 of the 13 patients had the tumor situated in the left kidney. One tumor was described as Angiolipoleioma of the kidney (case 13) and another (case 6) was described to be a highly differentiated carcinoma of urothelial type infiltrating the medulla of the kidney.

An important result is the overall five-fold increase of the EGF-receptor content in cancerous tissue (44 +/− 24 fmoles/mg protein) over the level found in the surrounding presumably tumor free tissue (6.4 +/− 4.2 fmoles/mg protein). There is no significant increase in

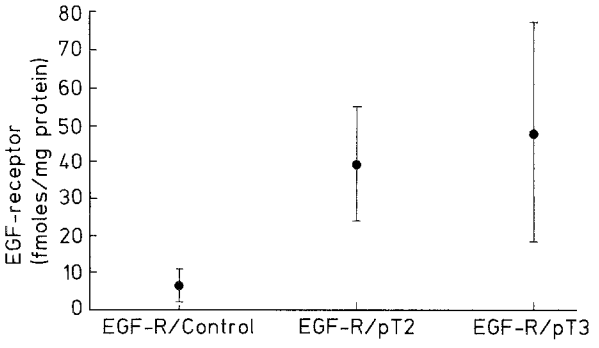

Fig. 1. EGF-receptor and tumor stages

EGF-receptor going from pT2 to pT3 stages (TNM-system of tumor classification, Fig. 1). However, measurements of EGF-receptor in necrotic parts of the tumors showed levels in the range of normal tissue (cases 3 and 4). In addition, in one patient (case 4) a metastasis to the bone could be retrieved and showed no evidence for EGF-receptor in the presence of relatively high background levels.

During the period of observation two patients died from their ailment (case 3 and 12). In case 3 the primary tumor was highly necrotic and had metastasized to the lung. In case 12 the highest level of EGF-receptor was detected. With an EGF-receptor level of 18 fmoles/mg protein the surrounding tissue was clearly already infiltrated by tumor cells.

Growth factors in serum and tissue

Using the radioimmunoassay for EGF and the enzyme linked immunoassay (ELISA) for TNF we measured the serum levels of the respective tissue hormones. For the EGF-RIA a normal range of 0.067 to 0.251 nmol/l is given by the manufacturer. The sera tested in this study showed an average level of 0.15 +/− 0.13 nmol/l for the presurgery EGF serum levels, whereas the serum levels of the post surgery patients showed EGF concentrations of 0.19 +/− 0.09 nmol/l. For TNF levels in normal sera we usually did not detect TNF. In our study most sera showed no detectable TNF. However, in some samples, strongly elevated levels were found (Table 1).

For some of the tumors it was possible to measure EGF in the cytosol prepared in a buffer containing protease inhibiting substances like EDTA and, interestingly, the tissue surrounding the tumor contained detectable levels of EGF whereas in the tumor tissue itself we were not able to show appreciable levels of EGF.

Discussion

Adenocarcinomas of the kidney have not been extensively analyzed by biochemical means. No tumor markers are currently available to detect this carcinoma at an early stage and no substances are known to be shed into circulation. Therefore, the clinician is limited to the removal of tissue at an early stage of the malignancy and it is important to gain insight into the prognosis of the malignant growth. Reports on amplified oncogenes such as N-myc becoming indicators of the stage of tumor growth in neuroblastoma [3] and as well as on oncogene amplification in lung tumors [17] indicate the value of such experiments in exploiting the potential of oncogenes and their products. The EGF-receptor is such an example of a protooncogene product. However, increased rates of gene transcription and not their amplification might be the reason for increased EGF-receptor expression in tumorous tissue [19].

We chose the quantitative technique to study EGF levels as immunocytochemical techniques [1, 17] yield more qualitative results and therefore are heavily dependent on the observer.

As we show here a significantly stronger expression of the proto-oncogene product, the EGF-receptor, in adenomatous carcinoma tissue in the kidney compared with the surrounding presumably normal tissue suggests that the EGF-receptor may be a useful tool in determining the stage of the tumor.

Although as indicated in Table 1, most of the tissue samples examined showed levels in the range of 30 fmoles/mg protein, well above the average 6 fmoles/mg protein found in normal tissue, benign lesions or necrotic tissue. There is a further level of EGF-receptor in the range of 50 to 100 fmoles/mg suggesting a steady increase of malignancy. An attempt to correlate the EGF-receptor levels, classified in such a way, with the histological stage fails however. Therefore we intend to further analyze these patients in order to reassess the value of EGF-receptor levels in the prognosis of the hypernephromas.

An interesting finding of the present work was the low level of EGF-receptor found in the urothelial carcinoma which had invaded the surrounding renal tissue. This contrasts with results reported by Berger et al. [1] who studied superficial bladder tumors with urothelial origin having no invasive potential and, therefore, no EGF-receptor gene amplification; on the other hand a single case of invasive bladder tumor had a significant increase of EGF-receptor gene expression. It will be the goal of further work to assess the role of the EGF-receptor as an indicator of classification of renal tumors.

Epidermal growth factor in serum was tentatively tested for its value as an indicator of the progress of tumor growth. As can be seen, there was no difference between pre- and post-surgical EGF-serum levels. It is of interest, however, to mention the results on the EGF-levels in tissue expressed on the basis of soluble protein. In most of the tissues examined the EGF-level was too low to be detected. In some samples tested the corresponding normal tissue surrounding the tumor showed elevated levels of tissue EGF. This is true for malignant tissue as well as for benign tissue (case 13, Table 1). Since serum levels of EGF are much higher on a mole to mole basis, we do not know the basis for this finding.

Tumor necrosis factor (TNF) is reported to increase EGF-receptor expression in fibroblast tissue cultures [12]. We examined the material available whether a correlation of TNF levels in tissue extracts and serum exists to the EGF-receptor levels. As serum levels of TNF tend to be extremely low, any increase of the concentration of this growth factor must be of somes significance. Unfortunately the number of samples tested was too small to establish any correlation with tumor stage invasiveness or the level of EGF-receptor, with the exception that the invasive urothelial kidney tumor (case 6) showed the highest level of serum TNF.

EGF-receptor levels in steroid hormone dependent tumors such as breast cancer have been reported as inversely correlated to the steroid hormone receptor level [7, 14]. We studied androgen receptor levels in both tumorous and surrounding kidney tissue with the aim to correlate this level with the EGF-receptor levels. No androgen receptor could be detected in kidney tissue (results not shown). This does not come as a surprise as kidney is not known as steroid hormone dependent organ.

Further work will be required to establish the value of the EGF-receptor as a diagnostic tool in determining tumor stage in a manner less subjective than that provided by current methodology.

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