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RELATION BETWEEN ER AND EGFR IN PRIMARY HUMAN BREAST CANCER
reports at variance

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Recently our laboratory developed a hyroxylapatite assay for measuring epidermal growth factor receptors (EGFR) in human tissue. (Benraad et al.) In a pilot study we analysed 67 breast tumour specimens for estrogen-receptor (ER) and epidermal growth factor receptor content. We found a strong negative correlation between ER and EGFR levels, Spearman rank-correlation coefficient -0.566 ($p<0.001$, DF=65).
A survey of the current receptor literature led to the following result:

Author	n	EGFR +		EGFR -	
		ER +	ER -	ER +	ER -
Battaglia 1988	55	12(22)	11(19)	24(44)	8 (15)
Delarue 1988	100	10(10)	12(12)	66(66)	12(12)
Pérez 1984	95	16(17)	24(25)	32(34)	23(24)
Rios 1988	225	29(13)	67(30)	72(32)	57(25)
Sainsbury 1987	135	4(3)	43(32)	60(44)	28(21)
This study1990	67	22(33)	21(31)	24(36)	0(0)

(n=number of patients on studie, ( )=% off n)

From the variance of the results listed above, most strikingly displayed in the EGFR+/ER+ and the EGFR-/ER- groups, we conclude that to enable future comparison of clinical evaluations the use of a standardized technique with strict assay conditions, already common for ER and PgR, to measure EGFR is of the utmost importance.

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EPIDERMAL GROWTH FACTOR IN HUMAN BREAST CYST FLUID. RELATIONSHIP TO CATION-RELATED CYST SUBPOPULATIONS.

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Epidermal Growth Factor (EGF) was evaluated in 102 specimens of breast cyst fluid (BCF), obtained by fine needle aspiration from 102 premenopausal women bearing mammary macrocysts. EGF was measured by RIA kit (Amersham, U.K.). Intracystic levels of K^+ and Na^+ were also evaluated. According to the cationic pattern, cysts were divided in 3 subgroups: type I, $K^+/Na^+ > 1.5$ (64 samples); type II, $K^+/Na^+ < 0.66$ (24 samples); type III, K^+/Na^+ 0.66-1.5 (14 samples). EGF was assayable at appreciable concentrations in all samples examined (range 3.2-246 ng/ml; median 47.2 ng/ml). EGF levels appeared directly correlated with the K^+/Na^+ ratio (Spearman's rank correlation, $p < 0.001$). When comparing intracystic concentrations with the cation-related subsets of cysts, EGF was significantly higher in type I vs type II cysts (median type I, 61.15 ng/ml; median type II, 30.1 ng/ml; Mann-Whitney U-test, $p < 0.005$). The omnipresence of EGF in BCF, a medium containing various biologically active substances such as steroid hormones and proteins, suggests a possible interaction of hormonal and paracrine regulators within the mammary gland. It is well-known that type I cysts show the highest concentrations of steroids (both androgens and estrogens) and are more frequently associated with proliferative lesions in the surrounding mammary tissue such as apocrine metaplasia and atypical hyperplasia. When considering that EGF seems to play a major role in the autocrine and paracrine regulation of the human breast cancer, the higher concentrations found in type I fluids focus on this subset of cysts as a possible marker for increased breast cancer risk. In this sense prospective cohort study are now ongoing both in Italy and U. K.

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A QUANTIFICATION OF IMMUNOCYTOCHEMICAL ASSAYS OF RECEPTORS FOR STEROID HORMONES AND GROWTH FACTORS

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The immunoreactivity of the receptors for estrogen (ER) progesterone (PR) and epidermal growth factor (EGF-R) were compared with the quantitative receptor-analysis, to examine the accuracy of immunocytochemical assays (ICAs). The ER-ICA and PR-ICA (Abbott) were performed on frozen sections (4 µm) of 92 breast carcinomas. After preparation of the breast-tumor cytosolic fractions, ER and PR were also determined by a dextran-coated charcoal (DCC) binding assay (alpha-16-(125)J-3,17ß-estradiol; gestagen (3)H-R 5020, DRG Biochemie) in tumor cytosol simultaneously. The EGF-R-analyses were done in 15 breast carcinomas, by an ICA (anti-mEGF-R, Amersham) and in the fractions of the tumorcell membranes by an radioligand binding assay (RLBA, (125)J-EGF, NEN). A light microscopic "immunoscore" (IS), ranging from zero (minimum) to 12 (maximum) was used; the receptorbinding capacities were expressed in fmol/mg cytosolprotein. The coefficients of correlation (ICA vs DCC) were: ER $r > 0.88$ ($p < 0.01$), PR $r > 0.96$ ($p < 0.01$) and EGF-R (ICA vs RLBA) $r > 0.89$ ($p < 0.01$). The sensitivities were: ER-ICA 91.1%, PR-ICA 93.2% and EGF-R 71,4%, the specificities were: ER-ICA 93.1%, PR-ICA 100% and EGF-R 87,5%. The positive predictive values (PVs) were: ER 96.2%, PR 100% and EGF-R 83,3%, the negative PVs were: ER 84,4%, PR 85.7% and EGF-R 77,8%. The immunocytochemical receptor analyses are comparable with the biochemical assays up to 500 fmol/mg. The maximal IS can not be used for the determination of the absolute receptor concentration. For the biochemical assays, the cut-off levels for ER and PR are ≤ 10 fmol/mg and for EGF-R ≤ 15 fmol/mg. The IS = 1 for ER corresponds to 9.7 fmol/mg, for PR 8.7 fmol/mg and the IS = 2 for EGF-R to 15 fmol/mg. These are the cut-off values of the "immunoscores" of the immunocytochemical assays.

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1,25 Dihydroxyvitamin D3 [1,25$(OH)_2$ D3] INCREASES THE GENE EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGF-R) IN THE HUMAN BREAST CANCER CELL LINE BT-20

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The effects of 1,25$(OH)_2$ D3 on EGF-R regulation were investigated on the human breast cancer cell line BT-20. These cells, devoid of sex steroids receptors, with known amplification of the EGF-R gene, display a great number of EGF-R ($1 \pm 0.4 \times 10^6$ sites per cell) with two class of sites : one of high affinity (Kd=0.48 ± 0.2nM) the other of low affinity and higher capacity (Kd=2.24 ± 0.93nM). Biological assay and quantitative election microscopy autoradiography combined with iodinated ligand binding to specific receptors demonstrated that the number of binding sites per unit of length of plasma membrane was 2.48 fold higher in treated than in control cells. This effect is not due to modification of the EGF internalization and degradation processes. Inhibition of the effect of 1,25$(OH)_2$ D3 by cycloheximide suggests that it is dependent on new protein synthesis.
EGF-R gene expression was investigated by the Northern method using human EGF-R cDNA probe and by in situ hybridization. EGF-R mRNA is found to be over expressed, the degree of stimulation is related to the dose : the threshold of sensitivity is observed at 10^{-10} M and in maximum at 10^{-9} M.
The present investigation indicates that 1,25$(OH)_2$ D3 up regulates EGF-R mRNA in BT-20 cells.