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IDENTIFICATION OF GENES INVOLVED IN DEVELOPMENT OF HORMONE INDEPENDENCE IN HUMAN BREAST CANCER.

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Growth of breast cancer cells is regulated by steroid hormones and can be blocked by hormone antagonist in one-third of the clinical cases. A major problem of endocrine therapy is that eventually all tumors become unresponsive. Our recent experiments show that hormone-dependence in human breast cancer cells (ZR-75-1) can be completely bypassed by introduction of the human Epidermal Growth Factor-Receptor (EGF-R) cDNA. These results indicate that single genetic perturbations may induce hormone-independent growth. Therefore we have initiated a random search for genes capable of sustaining growth in the absence of estradiol.

Retroviral DNA integration in the host genome is considered to be a largely random process and may result in (in)activation of gene expression. Large numbers of ZR-75-1 cells have been infected with defective, amphotropic retroviruses and subsequently cultured in the presence of hydroxy-tamoxifen. Proliferating cell clones have been isolated from these cultures and are currently being investigated for common retroviral DNA integration sites. This involves Southern analysis, in vitro DNA amplification using the Polymerase Chain Reaction and molecular cloning. This novel approach may lead to the identification of regulators of human breast cancer cell growth.

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EGF RECEPTORS IN NONAFFECTED AND TUMOROUS DOG MAMMARY TISSUES

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Growth and differentiation of mammary tissue is controlled, amongst others, by steroid hormones and various polypeptide growth factors. In the dog the presence of receptors for estrogens (ER), progestins (PR) and prolactin has been found to be much less common in mammary cancers than in nonmalignant tissues, suggesting a more autonomous growth in the former. In the present study we examined the expression of EGF receptors (EGF-R) in relation to the histopathologic state of transformation of dog mammary tissue. EGF-R analysis was performed by a multiconcentration radioreceptor assay in nonaffected mammary glands (NMG), in benign (BT) or malignant primary tumors (MT-I), and in metastatic tumours (MT-MET). In MT-I a division was made between samples of tumor mixed with pre-existing mammary epithelium (PME+) and those without (PME-).

In this limited series no significant variation was found in the presence or concentration of EGF-R (expressed as fmol/mg membrane protein) amongst the different tissues (see Table).

Tissue	n	EGF-R+	EGF-R concentration (median)	(range)
NMG	5	3	6.2	0- 60.4
BT	8	7	19.0	0- 96.6
MT-I, PME-	17	13	15.9	0-209.1
MT-I, PME+	13	7	7.6	0- 39.0
MT-MET	10	6	15.2	0- 61.6

No significant relation was found in these tissues between the presence of EGF-R and that of cytosolic ER or PR (not shown).

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RELATIONSHIP OF THE EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) WITH THE GROWTH FRACTION (GF) AND THE FLOW CYTOMETRIC S-PHASE (CSP) AS CELL KINETICS PARAMETERS IN HUMAN MAMMARY CARCINOMAS (HMC).

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EGF has been shown to have a mitogenic effect on some breast cancer cell lines in vitro and in vivo the growth of the HMC expressing the EGFR seems to be mediated by autocrine mechanisms rather than steroid hormones. The EGFR status as detected by an immunocytochemical method, was compared with the GF by the Ki-67 antibody and the CSP content as tumor proliferative indexes, and with DNA ploidy in 86 stage I-II HMC. Overall 52 out of 86 (60%) of the tumors were EGFR positive. There were 61 (71%) Ki-67 immunoreactive tumors (range: 3% to 60%) with a mean value of 15% nuclei stained. The 27 (32%) tumors with values above the mean were considered high proliferating. The proportion of CSP ranged from 2% to 32% with a mean value of 14% (cut-off value) 39 (45%) tumors were high proliferating. No correlation was found between cell kinetics parameters and EGFR status, suggesting that these are independent variables. In our series 57% of tumors were DNA aneuploid and only a trend was found with EGFR positivity ($p=0.08$). These results suggest the possibility of recognizing subsets of patients with diverse tumor aggressiveness, combining together EGFR status, cell kinetics and ploidy, for a better stratification of treatment options.

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EVALUATION OF EPIDERMAL GROWTH FACTOR RECEPTOR OCCUPENCY BY EGF-LIKE PEPTIDE IN 55 BREAST AND 42 NON BREAST TUMOR BIOPSIES

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EGF is one of several growth factors (EGF) involved in normal epithelial development and its receptor has been reported to have potential as a pronostic indicator in breast cancer. Moreover experimental data indicate that a large proportion of breast carcinoma produce TGFα. Since this growth factor binds EGF-R we have investigated if the separate measurement of non occupied ($EGF\text{-}R_1$) and total ($EGF\text{-}R_2$) binding sites might provide more complete data. A preliminary study has been performed in a series of 55 breast tumor biopsies and 42 samples taken in tumors of various origin and not usually considered as hormonedependent. EGF-R was determined in the crude membrane fraction of tumors by an [^{125}I] binding assay. Non occupied and total EGF binding sites were separately evaluated in each sample by an appropriate method. Estrogen receptors (ER) were measured by the DDC method. Results $>$ 10 fmoles/mg of membrane or cytosol protein were considered as positive.

In the breast tumors series we found 13/20 and 13/35 $EGF\text{-}R_1$ versus 15/20 and 27/35 $EGF\text{-}R_2$ in the ER- and ER+ tumors respectively. Therefore the total rate of $EGF\text{-}R_2$ positivity was 75 and 77% in ER- and ER+ tumors respectively.

In the other tumors the $EGF\text{-}R_1$ and $EGF\text{-}R_2$ positivity were 59 and 78% respectively.

These data indicate that EGF-R are present in $\simeq$ 75% of 97 tumors. The ER+ breast tumor dysplays the highest proportion of these sites occupied by an EGF-like endogenous peptide.