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ESTROGENS REGULATE c-erbB-2 ONCOGENE EXPRESSION IN NORMAL AND NEOPLASTIC MAMMARY CELLS.

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Amplification and overexpression of the c-erbB-2 oncogene, detectable in 20-30% of primary breast tumors, are related to breast cancer aggressiveness, as reported by a number of studies.

We have investigated the possible relationship between hormonal stimulation and c-erbB-2 expression "in vitro" and "in vivo". In T47D and MCF7 human breast cancer cells, as determined by both Northern and Western blot analysis, estrogens strongly and rapidly depressed c-erbB-2 expression. Conversely, c-erbB-2 was dramatically stimulated in growth-arrested cells. c-erbB-2 inhibition by estrogens was then studied using the estrogen and prolactin- dependent MTW9 transplantable rat mammary tumor model. The c-erbB-2-encoded p185 level was found strongly enhanced in tumors regressing after hormone withdrawal, whereas it was almost undetectable in growing tumors.

Estrogen regulation of c-erbB-2 appears to represent a more general physiological phenomenon. In fact, in the normal rat mammary gland, we observed that p185 is expressed exclusively in the fully differentiated tissue. i.e. at the end of pregnancy and during lactation.

Our data provide evidence that estrogens inhibit c-erbB-2 expression in mammary cells. c-erbB-2 amplification and/or overexpression in breast cancer may then signal the cell escape from estrogen control.

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c-erbB-2 ONCOGENE EXPRESSION CORRELATES WITH THE ABSENCE OF PROGESTERONE RECEPTORS AND HAS PROGNOSTIC VALUE IN BREAST CANCER.

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The expression of c-erbB-2 oncogene was evaluated by immunoblotting analysis of the encoded p185 protein on 133 human primary breast carcinomas. The DNA obtained from 63 of these samples permitted the analysis of c-erbB-2 genomic status by Southern blotting. p185 was observed in 51 samples (39%), whereas amplification of c-erbB-2 was found in 22% of the samples. Even though 20 samples with no detectable amplification expressed p185, expression and amplification of c-erbB-2 were significantly correlated ($P<0.007$). Expression of p185 correlated significantly with overexpression of the ras oncogene p21 protein, analyzed on the same tumor lysates by immunoblotting ($P<0.0006$). Expression of p185 was not associated with lymph node involvement, but it was significantly correlated to the absence of progesterone receptors (PgR) ($P<0.04$).

Follow-up data analysis showed that the presence of p185 in tumor lysates is predictive of short-term relapse and death. Interestingly, the predictive power of p185 seems limited to PgR+ tumors.

Our data suggest that the expression of c-erbB-2 p185 protein may be a valuable prognostic parameter for low-risk breast cancer patients.

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EARLY RELAPSE IN HUMAN BREAST CANCER IN RELATION TO BOTH c-erbB-2 OVEREXPRESSION AND ESTROGEN RECEPTOR STATUS.

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The knowledge of estrogen receptor (ER) levels in human breast cancer constitutes a controversial prognostic factor. Among ER^+ patients regarded as having a more favorable prognosis than ER^- patients, we have recently defined a group presenting a high risk of relapse. This group named $ER^+[R_2]$ is characterized by a ratio ER-protein (in fmol/mg protein) over ERmRNA (in pg/4µg total RNA) higher than 1.5. According to these results $ER^+[R]$ status has been proposed as an early prognostic factor (May et al. 1989, Oncogene, 4,1037-1042). In an attempt to correlate the prognostic significance of$ER^+[R]$ status with c-erbB-2 expression we have determined by Northern blotting the level of c-erbB-2 specific mRNA in the series of 89 untreated breast cancer previously analysed for ER specific mRNA. Over expression of c-erbB-2 mRNA (>20 pg/4µg total RNA) was positively correlated with

i) inflammatory carcinoma ($p=0.007$)
ii) lymph-nodes involvement ($p=0.03$)
iii)ER^- tumors ($p=0.02$).

A multivariate analysis with a median follow-up time of 30 months permitted the identification of the following independent predictors of early relapse : c-erbB-2 overexpression ($p=0.02$), ER^- status ($p=0.01$) and ER^+ [R] status ($p=0.01$). Moreover we can identify among ER^- patients regarded as having a less favorable prognosis than ER^+ patients, a group presenting a low risk of early relapse when c-erbB-2 mRNA is not over expressed.

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MULTIPARAMETER FLOW CYTOMETRIC QUANTITATION OF EPIDERMAL GROWTH FACTOR RECEPTOR AND C-ERB-B2 ONCOPROTEIN IN NEOPLASTIC AND NON-NEOPLASTIC TISSUES IN THE FEMALE GENITAL TRACT

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In this pilot study we assessed the novel use of multiparameter flow cytometry (FCM) to quantify the expression of epidermal growth factor receptor (EGFr) and C-ERB-B2 oncoprotein in fresh tissues and in blocks of tissue that were taken at the same time, frozen in liquid nitrogen and stored at -70° C. There is good corroborative evidence that alterations in the expression of these membrane oncoproteins may play a role in malignant transformation and tumorigenesis. EGFr and C-ERB-B2 expression in the female genital tract was measured by FCM in 24 normal tissues (ovary 8; endometrium 6; cervix 10) and in 21 carcinomas (ovary 6; endometrium 5, cervix 10). Cell suspensions were prepared by mechanical disaggregation of the fresh and thawed cryopreserved tissue samples. Indirect fluorescence was used to identify the oncoproteins and DNA was stained by propidium iodide.

High quality histograms were obtained and there was excellent correlation of oncoprotein measurements between fresh tissue and fresh frozen blocks of tissue (r 0.87). EGFr and C-ERB-B2 expression was significantly higher in the malignant tumours (103, SD 88 arbitrary fluorescence units and 256, SD 103 units respectively) than in normal tissue (40, SD 41 units and 89, SD 59 units respectively) ($p = 0.014$ and $p < 0.001$ respectively). A similar difference was found when cervical tissue alone was examined ($p = 0.04$ and $p = 0.005$ respectively). The highest expression of EGFR and C-ERB-B2 occurred in the S-phase of the cell cycle. Multiparameter FCM proved to be a promising technique for the study of membrane oncoproteins in fresh tissues and fresh frozen blocks of tissue.