

human mammary cancer cell lines. This element contains the recognition sequence for three known transcription factors: AP2, NFkB and ETF. Preliminary experiments indicate that neither factor is responsible for c-erbB2 overexpression through this newly identified sequence. Site directed mutagenesis experiments have identified the nucleotides which are important in transactivation.

Luciferase-reporter vectors, containing the new cis element in front of the c-erbB2 minimal promoter, were constructed and transfected into BT474 cells. The transcriptional activity of the new vector was comparable to that of the native promoter fragment, showing that the new sequence does contribute to c-erbB2 overexpression in vivo. Site directed mutagenesis of the cis sequence inhibited the transcriptional activity of the promoter fragment containing it. These experiments show that the factor recognizing this new cis element is important in c-erbB2 gene overexpression in BT474 cells. We are currently realizing the experiments aimed at the identification of the transcription factor recognizing this cis element, in order to understand the mechanism leading to the oncogene's overexpression in breast cancers.

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### Neu differentiation factor (NDF/hereregulin) induces expression of adhesion molecules, increased cellular invasiveness, and is expressed in Paget's disease of the breast together with its receptors

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**Purpose:** This study concentrated on the biological activity of NDF, a ligand to the erbB receptor family, on the migration of breast cancer cells to the epidermis in breast cancer cells and Paget's disease of the breast. NDF binds to erbB-2, erbB-3 and erbB-4 receptor tyrosine kinases and induces either cell growth or differentiation.

**Methods:** We used immunohistochemistry combined with quantitative image analysis, or the polymerase chain reaction (PCR).

**Results:** NDF induced integrin molecules; specifically  $\beta_1$ ,  $\alpha_2$ ,  $\alpha_3$ ,  $\alpha_5$ , the adhesion molecule CD44. This was accompanied by increased cellular invasiveness. Since NDF contributes to the invasiveness of cancer cells, we investigated the presence of erbB family receptors and NDF. All specimens overexpressed erbB-1, erbB-2, erbB-3, erbB-4 and NDF and integrins associated with migration of cancer cells and keratinocytes.

**Conclusion:** An autocrine loop involving NDF and its receptors may be operative in Paget's disease of the breast, contributing to their invasive characteristics of the cancer cells.

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### Multisite phosphotyping of the ErbB2 oncoprotein in 102 human breast cancers

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**Purpose:** The commonest receptor tyrosine kinase overexpressed in human tumours is the unliganded ErbB2. By analyzing patterns of multisite phosphorylation within tumour-associated ErbB2, we have aimed to clarify its pathogenetic role and clinical significance.

**Methods:** A panel of reagents has been prepared which permits selective analysis of differentially phosphorylated ErbB2 isoforms. Conventional experimental techniques were used to establish the in vitro significance of these isoforms, while clinical correlations were sought to determine their prognostic implications.

**Results:** In vitro studies implicate ErbB2 as a molecule which amplifies the signalling of ambient growth factors by preventing the downregulation of heterodimerized receptors. Multisite ErbB2 phosphotyping was approximately twice as sensitive as conventional immunohistochemical staining for ErbB2 expression, indicating that up to 60% of human breast tumours 'oversignal' via ErbB2. In a small cohort of patients with accessible clinical information, survival was far poorer in the presence of a detectable Tyr<sup>1248</sup> or Ser<sup>1113</sup> phosphorylation signal.

**Conclusion:** ErbB2 phosphotyping contributes more information as to tumour biology and prognosis than does traditional immunohistochemical analysis, suggesting a new dimension in the molecular characterization of human tissues.

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### Mutations in the p53 gene, both within and outside the zinc-binding domains, are associated with increased apoptosis and mitosis in invasive breast carcinomas

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In breast cancer, mutations located in the zinc-binding functional domains of the p53 gene have been associated with worse prognosis and worse response to treatment with doxorubicin, compared to mutations in other parts within exons 5-8 of the gene. To investigate whether these specific p53 mutations are associated with differences in rate of apoptosis and/or mitosis or expression of the anti-apoptotic Bcl-2 protein, we evaluated these parameters in 93 invasive breast cancers with a confirmed p53 mutation in exon 5-8 and in 99 tumors with no p53 mutation. Apoptotic cells were identified by morphological criteria and staining of DNA strand breaks (TUNEL-assay). Tumors with mutations in the zinc binding domains did not differ in their mitotic or apoptotic activity from tumors with mutations outside these domains. However, compared to the wild type p53 tumors, both mean apoptotic and mitotic levels were approximately two-fold increased in the mutant p53 group ( $p < 0.001$ ). Presence of a p53 mutation was also associated with necrosis ( $p < 0.001$ ), high tumor grade ( $p < 0.001$ ) and low expression of Bcl-2 ( $p < 0.001$ ). Accumulation of cellular p53 as detected by immunohistochemistry (IHC) correlated strongly with the presence of missense mutations and, with few exceptions, tumors with p53 frameshift or stop codon mutations were completely negative after IHC. Our data support the concept that in invasive breast carcinoma loss of p53 function is involved in enhanced proliferation rather than decreased apoptosis and that the resulting acceleration of cell turnover may enhance clonal evolution and tumor progression.

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### ICAM-1 up-regulation expression on breast cancer cell lines after all-trans retinoic acid (ATRA) treatment is independent of hormonal receptor conditions

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Intercellular adhesion molecule-1 (ICAM-1, CD54) is referred as playing a key role in the multiple interactions between tumor cells and the effectors of the immune system. Recent reports on defective or reduced expression of ICAM-1 molecule on breast cancer tissues can be linked to the impaired cytotoxic effects observed in vivo. The reduced expression of ICAM-1 constitutes a potential mechanism by which breast cancer cells escape immunologic recognition and lysis by the appropriate effector cells. ATRA is currently used in many clinical trials because of its ability to induce growth inhibition, while in breast cancer it apparently acts by favoring chemoprevention and differentiation. These observations led us to investigate the effects of ATRA on surface expression of ICAM-1 in three breast cancer cell lines, two of them hormone-responsive (T47D, MCF-7) and one non hormone-responsive (MB-MDA-231).

The results showed that: i) ICAM-1 expression is increased in all three cell lines and ii) the increment is observed independently of their hormonal receptorial status. Lastly, iii) ICAM-1 up-modulation is time- and dose-dependent and is reversible. Other differentiating and proliferating agents tested, e.g. DMSO, progesterone, estradiol or dexamethasone, do not exert detectable effects on the molecule.

The inferences derived from these results suggest that i) the effects mediated by ATRA include a significant and reproducible up-modulation of ICAM-1. Moreover, ii) ICAM-1 could consequently represent a mechanism through which ATRA acts by improving the endogenous cellular defenses against tumor. iii) Further relevance for in vivo treatment is that the up-regulatory effects are not linked to the expression of the estrogen and progesterone receptors.