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Expression of the Ets transcription factor family in HER2/ neu positive breast cancer

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Breast cancers, which over express the growth factor receptor, HER2/ neu, have a poorer prognosis. Activation of the Ets transcription factors, Ets-1, Ets-2 and PEA3 are implicated in the transcriptional regulation of HER2/ neu. Transcriptional activity of the Ets family is thought to be modulated by nuclear regulatory proteins, including amplified in breast cancer 1 (AIB1) and steroid receptor co-activator 1 (SRC-1).

Our aims were to localise these transcription factors and AIB-1 and SRC-1 in breast cancer using immunohistochemistry and to assess their co-expression using immunofluoresence. The ability of the growth factors bFGF and EGF to modulate the protein expression of the Ets transcription factors in primary breast cultures and breast cancer cell lines using western blotting was also assessed.

PEA3, Ets-1, Ets-2, AIB-1 and SRC-1 were localised within breast cancer tissue. The transcription factors and their co-regulators were co-localised to the same cell within breast cancer tissue. The growth factors bFGF and EGF induced an up-regulation in the protein expression of the Ets transcription factors in both the primary breast cultures and the breast cancer cell lines in a dose dependant manner. The growth factors, bFGF and EGF regulate the Ets family of transcription factors. Co-localisation of Ets with their co-regulators implicates nuclear regulatory protein interaction in the modulation of Ets activity in human breast cancer.

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Determination of HER2 gene amplification: validating Chromogenic in-situ hybridization (CISH) against IHC and FISH.

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The human epidermal growth factor receptor-2 (HER2) is amplified/overexpressed in 15-25% of human breast cancers. A positive HER2 status is associated with a poor prognosis, including reduced disease-free and overall survival, and current evidence indicates that HER2 may also be predictive of response to anticancer therapy. Of particular note, a HER2-positive status predicts for response to the anti-HER2 monoclonal antibody therapy, Herceptin® (trastuzumab). Moreover, the clinical benefits achieved with Herceptin® therapy are greatest in patients with high-level HER2 overexpression or HER2 gene amplification. Thus, accurate assessment of HER2 status is essential to identify those patients eligible for therapy. Immunohistochemistry (IHC), which determines the level of HER2 receptor overexpression is used most frequently. In most HER2 testing algorithms, fluorescence in-situ hybridisation (FISH), which measures the level of HER2 gene amplification, is used when IHC testing is not conclusive. FISH requires the use of a fluoresence microscope and identification of tumor cells is relatively difficult with this technique. Chromogenic in-situ hybridization (CISH) is very similar to FISH, but only requires a conventional light microscope and the tissue and cellular morphology can still be visualised. These are major advantages over FISH.

CISH is not currently validated for routine diagnostic HER2 testing. Therefore, a multicentre study has been initiated to compare the results achieved with CISH, IHC and FISH on formalin-fixed, paraffin-embedded breast tumour samples. This is a ring study in which five laboratories perform IHC, FISH and CISH on paraffin sections from 50 breast carcinomas from their own institute. In addition, unstained sections from the set of 50 cases from each laboratory are sent to one of the other participating laboratories where additional CISH testing is performed. The local IHC, FISH and CISH results will be compared with the CISH results obtained in the ring study. These data will be presented.

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Up-regulation of p27kip1 reduces matrix metalloproteinase 9 (MMP-9) in the breast cancer cell line resulting in inducing inhibition of invasiveness

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Background: p27kip1 is a cyclin-dependent kinase inhibitor which regulates progression of the cell cycle from G1 to S phase. Decreased expression of p27kip1 is observed in several cancers including breast cancer, and p27kip1 is one of prognostic factors in them. We previously reported that the over-expression of p27kip1 triggers apoptosis in several different human cancer cells. Recently, it was reported that p27kip1 relates to invasion, metastasis and angiogenesis in them, but its mechanism remains unknown. On the other hand, matrix metalloproteinases (MMPs) and integrins are very important proteins in tumor invasion and metastasis. We tried to find out the possibility of relation between p27kip1 and them.

Material and Methods: The breast cancer cell line, MDA-MB-231, and its p27kip1 stable transfectant (MDA-MB-231-27) were mainly used in this study. Invasion assay was done using Biocoat Matrigel invasion chambers. Furthermore western blots was done for changes of MMPs and integrins expression.

Results: There was not different in growth curve and viability between MDA-MB-231 and MDA-MB-231-27. MDA-MB-231-27 cells were significantly found to reduce the number of invasive cells in invasion assays ($p<0.05$). MMP-9 was decreased in MDA-MB-231-27 compared with parental cells. On the other hand, integrins expression is not confirmed in this study.

Conclusions: Up-regulation of p27kip1 resulted in suppressing invasion in the breast cancer cells and decreasing expression of MMP-9. The role of p27kip1 in tumor cell invasion is closely related with MMP-9 expression. In this study we report that p27kip1 plays a key role in tumor cell invasion and MMP-9 is involved with its phenomenon.

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Effect of herceptin on gene expression in her2 positive breast cancer cell line

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Her2/neu, an epidermal growth factor receptor mediates cell growth, differentiation and survival. Over expression of Her2/neu occurs in up to in 30% of breast cancers and its associated with a high risk of relapse and death. Herceptin is an anti-Her2neu monoclonal antibody that inhibits that particular receptor and it has been a valuable addition to the standard therapy demonstrating a survival benefit. Development of resistance to Herceptin treatment is common but not well understood. Insulin-like growth factor I receptor (IGF-IR), another member of the tyrosine kinase family, has been discussed with increased risk of several cancers. However, resistance to Herceptin might not be merely dependent on the lack of efficacy in inhibiting Her2/neu but might be associated with IGF-IR over expression. We investigated the anti-proliferative effect of Herceptin on a breast cancer model system using microarray assay. We also evaluated the effect of Herceptin on mRNA expression of IGF-IR. A Her2/neu positive cell line (SKBR3) was cultured with and without Herceptin. mRNA was extracted and analysed in a cell-cycle specific cDNA microarray. Expression of the IGF-IR gene was determined by applying solution phase RT-PCR and RNA in situ hybridisation. In situ hybridisation and RT-PCR showed similar expression patterns for IGF-IR with and without Herceptin. Herceptin has an anti-proliferative effect on the Her2neu cell line. Microarray technology is a useful tool to check the anti-proliferative effect of Herceptin on Her2/neu positive breast cancer cells. The association between IGF-IR and resistance to Herceptin need to be further evaluated.