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Serum soluble VCAM: A surrogate marker of angiogenesis

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Purpose: Angiogenesis is essential for tumour growth and metastasis. Vascular cell adhesion molecule-1 (VCAM) and endothelial-selectin (ESEL) are expressed on activated endothelial cells and we hypothesized that their measurement in serum would be an accurate measurement of tumour angiogenesis

Methods: Preoperative serum levels of VCAM, ESEL and VWF (another EC marker) were measured by enzyme-linked immunosorbent assay (ELISA) in 93 women with early breast cancer and levels were correlated with histological prognostic features and the microvessel density in each tumour (assessed by CD31 immunostaining). Sequential serum samples were taken from 55 women with advanced breast cancer, immediately prior to a change in hormonal therapy and 3 months later. Changes in serum VCAM, ESEL and VWF were compared to the response of the disease assessed by UICC criteria at 6 months.

Results: In early breast cancer serum VCAM, not ESEL or VWF, correlated with the microvessel density ($r = 0.66$, $p < 0.001$) in each tumour whilst in advanced disease serum VCAM levels, not ESEL or VWF, rose in those women whose disease progressed ($p < 0.001$) but levels remained unchanged or fell in those women whose disease remained stable or showed a partial response to therapy.

Conclusion: Serum VCAM is a surrogate marker of angiogenesis in breast cancer and its measurement may help in the assessment of antiangiogenic drugs currently in phase II trials.

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Ovarian hormones effect VEGF expression in breast cancer cells

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Purpose: VEGF is one of the most potent angiogenic cytokines, whose tumour levels correlate with a decreased relapse free survival. The effect of the ovarian hormones on breast cancer progression and timing of surgical intervention remain controversial. The purpose of this study was to address these issues by determining the effect of oestrogen and progesterone on cell growth and VEGF expression in MCF-7 breast cancer cells.

Methods: MCF-7 cells were grown in triplicate. To each set the following hormone combinations were added: Oestradiol, oestradiol and the equivalent of follicular levels of progesterone, oestradiol and luteal levels of progesterone, and controls with no added hormones. Each day the percentage increase in cell growth was counted using a histocytometer. The supernatant was collected and assayed for VEGF by quantitative ELISA.

Results: Oestrogen caused an increased cell growth and VEGF expression compared to controls (mean VEGF 650.06 vs 503.46 pg/ml; $p = 0.05$). The luteal combination of hormones resulted in less cell growth and VEGF expression compared to the follicular combination (mean VEGF – 481.02 vs 650.03 pg/ml; $p = 0.05$) (Mann-Whitney)

Conclusion: This is the first time the effect of the menstrual cycle hormones has been shown on VEGF expression on breast cancer cells. Lower VEGF expression with luteal levels of hormones may imply a lower metastatic potential in this phase of the cycle. This supports the evidence favouring the luteal phase for surgical intervention in premenopausal women and may have therapeutic implications in breast cancer management.

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Breast cancer tissue analysis by computerized bidimensional polyacrylamide gel electrophoresis (2DPAGE) and N-terminal microsequencing

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Purpose: An area of intense research in breast cancer (BCa) is the investigation of changes in gene expression associated to the neoplastic transformation. The identification of unknown cancer-related proteins may improve the knowledge of the biomolecular mechanisms involved in the pathogenesis of the neoplasia and lead to the detection of new tumour markers. In this study, 2DPAGE was used to obtain qualitative and quantitative information on protein expression in BCa.

Methods: Specimens of 12 ductal BCa and non-neoplastic adjacent tissues were analysed by 2DPAGE using the immobiline-polyacrylamide system. Proteins were identified either by N-terminal microanalysis, gel matching with reference maps or by a combination of these methods.

Results: The protein pattern of both neoplastic and non neoplastic breast tissues was similar, except for a set of 32 spots highly expressed in carcinomas, while less intense and occasionally undetectable in non-neoplastic mammary tissues. Spots were identified by image analysis and N-terminal microsequencing. Intriguingly, besides folding proteins and glycolytic enzymes, proteins related to cell proliferation and to the immune response were identified.

Conclusions: 2DPAGE combined with microanalysis seems to be a technique of choice to investigate gene expression in breast tumours. For BCa, a systematic characterisation of tumour proteins may bring to new insights in the biology of the disease and open the way to the identification of candidate tumour markers for diagnostic and prognostic purpose.

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Cytogenetic analysis of 112 breast tumors

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Background: In spite of the development of basic research, little is still known about the cytogenetic features of breast cancers. The great complexity of the interpretation of the results and to a certain extent, technical difficulties have been major obstacles to the development of this approach.

Design: 112 primary breast tumors were cytogenetically analysed. This series included 94 invasive ductal carcinomas (IDC), 8 invasive lobular carcinomas, 8 other subtypes of carcinomas and 2 cystosarcomas phyllodes (CP). The tumor tissues were dissociated mechanically and then enzymatically prior to cell culture (4 to 30 days).

Results: The percentage of failure was 13% (15 cases). Clonal chromosome aberrations were detected in 33 (41%) of the cases. The more common abnormalities of number were: +7, +8, +20, and −X. Among the structural alterations, loss of 1p, 1q, 3p, 3q, 6p, 6q, 7q, 8 p, 8q, 11p, 16q, Xp and gain of 1q, 3q, 6p, 8q were frequently reported (map of karyotypic imbalances). More specific alterations were: i(1q), t(1; 16)(q10; p10), del(3)(p12–13; p14–21), del(6)(p22–27). In the 3 male IDC, clonal abnormality of the sexual chromosomes occurred: −Y (2 case), +X (1 case). The 2 CP showed double minutes. In this series, clonal abnormalities were more frequent in women over 55 years ($p = 0.01$), particularly for chromosome 7 ($p = 0.03$). No correlation was observed between the presence of clonal alterations and different clinicopathological parameters.

Conclusions: These findings indicate that specific chromosomal regions are non randomly involved in breast tumors and we could identified karyotypic subgroups. At the light of these results, we now focus our attention to the cases with trisomy 7 and 8.

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Primary paclitaxel in breast cancer: Is beta-tubulin a predictor for pathological response?

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Purpose: A study of primary paclitaxel for T2 breast cancer and another study of paclitaxel and radiation as for locally advanced breast cancer are ongoing at our institution. Pre-treatment tumor biopsies are obtained to explore molecular determinant of pathological response to treatment. Among the molecular correlates studied, we analyzed the pattern of beta-tubulin (the main known target of action of paclitaxel) in the original specimens to explore preliminary correlations with pathological response induced by paclitaxel.

Methods: We analyzed tumor biopsy specimens obtained from 13 breast cancer patients prior to treatment with paclitaxel and radiation (8 patients) or primary paclitaxel (5 patients). PCR primers were designed as previously described for six tubulin isoforms (Kavallaris M et al. Clin. Cancer Res, 1997). These primers give at least a 2-log linear range of PCR amplification. Pathological response was evaluated at mastectomy based on the following classification: pCR = clearance of invasive cancer in the breast and axilla,