539 ORAL

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.

540 POSTER

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.

541 POSTER

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.

542 POSTER

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.

543 POSTER

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,

pPR = persistence of less than 10 microscopic foci of invasive tumor cells in the breast or in the axilla; pNR = any larger amount of residual cancer cells in the breast or in the axilla.

Results: Gene expression was plotted toward pathological response. Low tubulin levels in isoforms III, IVa, IVb were associated with pathological response.

Conclusion: The patterns of beta-tubulin isoforms distribution may predict for pathological response to paclitaxel and paclitaxel/radiation regimens.

544 POSTER

Proliferation, apoptosis and related markers in invasive ductal breast carcinoma

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Purpose & Methods: Expression of bcl-2, bax, p53 and PCNA genes was studied immunohistochemically in 170 invasive ductal breast carcinomas (median follow-up time 91 months, range 24–186 months). In addition the mitotic activity index (MAI) and apoptotic cell death (Tunnel technique) were scored. Classic histopathological features and steroid receptor status of the tumours, and clinical patient characteristics were incorporated in the database.

Results: No relationship could be observed between bcl-2, bax or p53 status and tumour grade, pTNM staging and menopausal status. A strong positive relationship was demonstrated between bcl-2 immunoreactivity and steroid receptor status (ER and PR: $p < 0.001$). There was an inverse relationship between bcl-2 expression and p53, but not with bax or PCNA. Multivariance analysis demonstrated absence of bcl-2 expression and the MAI to be independently related to shortened disease-free survival ($p < 0.01$) and shortened overall survival ($p < 0.001$).

Conclusions: Our data suggest that bcl-2 expression plays a crucial role in the behaviour of invasive ductal carcinoma and may be an important modulator of response to adjuvant therapy.

545 POSTER

Incidence of breast cancer associated with use of hormone replacement therapy and other risk factors in 1709 patients

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Background: Various studies have suggested an association between hormone replacement therapy (HRT) and breast cancer but the reports have been conflicting and controversial. A meta-analysis of 52 epidemiological studies in 21 countries by the Imperial Cancer Research Fund (ICRF) in 1997 concluded that the relative risk for breast cancer was 1.023 for each year of use.

Aims:

(1) To assess the incidence of HRT related breast cancer in our patient population.
(2) To study other risk factors in the women who developed breast cancer while on HRT.

Patients & Methods: A retrospective study of 1709 patients seen between January 1987 and December 1997 was made. The mode of presentation, duration of HRT, age at diagnosis, other "risk" factors (alcohol, smoking, family history, past history of breast biopsy) and pathological features of the cancer were analysed.

Results: Sixty-five per cent of the 62 patients (mean age 60 years) with HRT associated breast cancer presented with symptomatic disease while 19% were detected within the UK Breast Screening Programme. The duration of HRT intake was <2 years in 9, 2–5 years in 24, 6–10 years in 17, 10–15 years in 5 and >15 years in 5 patients. In 38.7% a family history of breast cancer (11 first degree and 13 second degree relatives) was noted. Smoking and alcohol intake was average in 87% of patients. Twenty patients had a history of a previous benign breast biopsy. Histological review showed that 55.8% had a T1 carcinoma and 57% were N0. Invasive ductal carcinoma was found in 82.3% with 19.5% being Grade I (Bloom & Richardson) and 61% Grade II.

Conclusion: The highest risk in relation to HRT appears to be in the first five years suggesting that HRT may stimulate the growth of an undetected cancer. The high association with a strong family history and previous benign biopsies has implications for women seeking advice about starting HRT.

546 POSTER

Breast cancer and missense mutations in the transactivation region of the BRCA1 gene

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Purpose: The implication of missense mutations in germline of BRCA1 gene in the pathogenesis of breast cancer is not well understood. When a missense mutation occurs in a specialized region of the gene, the functional impact of this change can be meaningful.

Methods: We investigated missense mutations located in the transcriptional transactivation gene region (amino acids 1528–1863), and correlate them with clinical and familial characteristics of the patients. We studied 192 patients, 87 with a definite family history of the disease, and 105 without antecedents and considered to have sporadic breast cancer. The entire coding region of the BRCA1 gene was analyzed by the PCR-SSCP method. Specimens showing a differential band were amplified and used for direct DNA sequencing.

Results: Two mutations were detected, Glu1735Lys and Asp1778His. The first mutation was identified in a family with five breast cancer patients in first-degree, distributed between two generations. One patient showed bilateral tumor. The second missense mutation appeared in a 44-year-old patient with a sporadic invasive ductal carcinoma with axillary involvement and negative steroid receptors.

Conclusions: The two new mutations detected may represent a functional change in the transactivation potential of the BRCA1 gene.

547 POSTER

The expression of new protein taking part in cancerogenesis, p65, and its correlation with steroid receptors in ductal carcinoma of female breast

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A 65-KD phosphoprotein (p65) was isolated from human breast carcinoma cell line MCF7. The aminoacid sequence analysis of N-terminal part of the p65 molecule was similar to the steroid receptor protein. This raises the possiblility that the p65 gene may belong to the family of genes which encode nuclear receptors for various hydrophobic ligands of steroid hormones. Paraffin-embeded tissue slides from 89 infiltrating ductal carcinoma specimens were assessed immunohistochemically with the usage of monoclonal antibodies against human p65 antigen. The p65 expression was correlated with oestrogen receptor (OR) and progesterone receptor (PR) levels and grade of malignancy according to Bloom and Richardson scale. It is suggested that the high OR and PR levels are accompanied by the presence of p65 in breast cancer tissue. The funcion of p65 and its ligands is still unknown. However, p65 may be important in the process of development of tumours. It is probable that the conserved cysteine-rich domains found in the human p65 and which are also common to human OR provide an important biological funcion.

548 POSTER

A prospective study on genetic risk factors in an unselected sample of breast cancer patients who receive adjuvant radiotherapy

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5–8% of all breast cancers (BCs) are due to hereditary factors. Detection of women at high risk for BC and offering genetic counselling (GC) to them might be worthwhile. In high risk women regular mammography (started at younger age) and preventive surgery (in some cases) may result in substantial gains in health and in life expectancy. Women at high risk for BC can be identified by systemetically searching for presumed risk factors. The Radiotherapy Department of the University Hospital (UH) in Utrecht together with the Clinical Genetics Center and the Comprehensive Cancer Center prepared a study to prospectively evaluate the prevalence of risk factors for hereditary BC in 1.000 patients. All BC patients referred for radiotherapy as part of curative treatment for their disease are included (60% of all newly