

medium containing EGF resulted in being the best growth of epithelial cells. Chromosomal abnormalities were found in all 20 tumors. The changes were clonal in 10 tumors (50%). Karyotypic analysis of first or second passage cultures yielded predominantly diploid cells. Among the clonal aberrations, clones with 15q deletion (q13–q15, q12–q15, q7) were observed in 4 cases. In addition, there were two clones involving the chromosome 1 (1p31, q21 deletion). In conclusion, the most common cytogenetic abnormalities in Korean women affect chromosome 15 compared to chromosome 1 in caucasian women.

PP-1-11 Transferrin Receptor (CD71) Expression by Human Breast Cancer Cells

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The expression of transferrin receptor by malignant cells was studied in cryostat tumor sections of 44 patients with breast cancer using MoAbs. HLA-I and II, HMFG1, adhesion molecules (CD29) were also estimated as well as tumor infiltration with leukocytes (CD45+), T-lymphocytes (CD7+) and macrophages (D11+, GHI/61+, MAC387+).

CD71 was detected in 66% of cases, HLA-I in 43%, HLA-Dr in 22%, CD29 in 64%, HMFG1 – in 66% of cases. In CD71-positive groups compared to CD71-negative tumor cells revealed a statistically significantly higher proportion HLA class I (66% (19/29) vs 0% (0/15), $p < 0.01$) and of HLA-Dr (31% (9/29) vs 6% (1/15), $p = 0.05$).

No associations of CD71 with other markers were detected. CD71+ and CD71- groups did not have statistically significant differences as regards tumor infiltration levels with T-cells.

PP-1-12 Chemotherapy-Induced Diversification of DNA Profiles in Breast Cancer

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DNA flow cytometry was used to estimate spontaneous and therapy-induced changes in DNA profiles of breast carcinomas by comparing the findings from pre-treatment core needle biopsies and resection samples. Spontaneous diversification from primary diploid tumor to aneuploid node metastases was a rather rare event occurring in only 2% of 45 cases, 38% (17/45) of which expressed p53 protein in immunohistology. However, following chemotherapy (4–6 cycles, FAC regimen) 7 out of 20 originally diploid tumors (35%) were found to contain aneuploid population(s). No such diversification (0/27) was observed after radiotherapy and/or endocrine treatment. Since an aneuploid DNA profile is considered to be associated with more aggressive tumor behavior, chemotherapy may not be beneficial in individual cases which express newly-developed, chemotherapy-induced aneuploid populations. Whether p53 protein expression and/or other factors may predict increased tumor instability remains unclear.

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PP-1-13 Breast Cancer Cell Mediated Control of Human Stromelysin 3 (ST3) Promoter Activity

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ST3 is a matrix metalloproteinase overexpressed in stromal fibroblasts at the tumour-stromal interface of invasive breast cancers (Basset et al. *Nature*, 1990; 348: 699–704) and levels of expression of its mRNA have been correlated with both degree of invasion of primary breast cancers and clinical outcome (Engel et al. *International Journal of Cancer*, 1994, 58 (6): 830–835). We have shown, in transient transfection assays, that breast cancer cell factors modulate ST3 promoter activity. To characterize further the factors important in ST3 gene expression, clones of NIH3T3 fibroblasts stably expressing the firefly luciferase reporter gene under the control of 2 different lengths (0.46 kB and 3.4 kB) of the 5' flanking sequence of the human ST3 gene were created.

Co-culture of the human breast cancer cell lines MCF-7 and MDA-MB231, but not ZR75, BT20 or T47D cell lines with the stable fibroblast clones resulted in a consistent 2–3 fold upregulation of luciferase activity in the clones driven by 3.4 kB but not 0.46 kB of the ST3 promoter. These data suggest that certain breast cancer cell lines either by a cell-cell contact or diffusible factor mechanism can switch on human ST3 promoter activity, and furthermore that the putative response element in the promoter can be found between 0.46 and 3.4 kB upstream of the transcription start site.

PP-1-14 Integrins $\alpha v \beta 1$ and $\alpha v \beta 5$ Function as Breast Cancer Cell Vitronectin Receptors

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The classical vitronectin receptor ($\alpha v \beta 3$) is absent from most breast cancer cells suggesting that an alternative integrin may subserve a similar role to this receptor. In order to examine this possibility we have studied the expression and composition of αv -containing heterodimers on a range of human breast cancer cell lines (including ZR75, BT20, MB468, MCF7, MDA231, BT474). FACS analysis with heterodimer-specific monoclonal antibodies (MAbs) revealed cell surface $\alpha v \beta 5$ on all lines examined, no expression of $\alpha v \beta 8$, expression of $\alpha v \beta 6$ on 1 out of 6 and low levels of $\alpha v \beta 3$ on 1 out of 6 of the cell lines (BT20 and MDA231 respectively). These results were confirmed by immunoprecipitation analysis of cell surface ¹²⁵I-iodinated cells. Using anti- αv MAb (P2W7) as the immunoprecipitating antibody a protein of similar size to $\beta 1$ was brought down in conjunction with the αv subunit in ZR75, MCF7 and MDA231 cells. The $\beta 1$ identity of this band was confirmed by immunodepletion analyses. Adhesion assays to immobilised vitronectin with ZR75 cells were performed in the absence and presence of anti- αv (17E6), anti- $\alpha v \beta 5$ (P1F6) and anti- $\beta 1$ (P4C10) MAbs. Adherence to 5 μ g/ml vitronectin (21%) was reduced by anti- αv to 6%. ($p \leq 0.01$) Anti- $\alpha v \beta 5$ and anti- $\beta 1$ alone did not reduce adherence (20–30%) but in combination reduced adherence to 6%.

These results show that in breast cancer cells $\alpha v \beta 1$ and $\alpha v \beta 5$ serve as major functional vitronectin receptors in the absence of $\alpha v \beta 3$.

PP-1-15 Is Cowden Disease Gene a Tumor Suppressor Gene or Not?

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Cowden disease (CD) or multiple hamartoma syndrome is a cancer associated genodermatosis with a dominant autosomal pattern of inheritance. It was named for the first patient described in 1963 by Llyod and Dennis.

Its clinical features include many abnormalities but the main characteristics are hamartoma of the skin, breast, thyroid, oral mucosa and intestinal epithelium. By performing linkage analysis in a total of 12 families, the gene for Cowden disease has been localized to 10q22–23 with a significant lod score of 8.92 at $\theta = 0.02$ with the marker D10S 573 [1].

It is observed that clinical features of CD are similar to certain manifestations of phakomatosis such as neurofibromatosis, Von Hippel-Lindau disease and tuberous sclerosis. Considering that the genes involved in these diseases behave as tumour suppressor genes, we can suppose that such a gene could be also involved in CD.

To begin to answer to this question, we performed loss of heterozygosity analysis on 10q, in 3 breast tumors [2 carcinomas, 1 adenofibroma], with markers located in Cowden disease gene region.

In the aim of searching an eventual involvement of the CD gene in sporadic breast cancer, 30 invasive breast carcinomas were also studied.

[1] Nelen et al. Localization of the gene for Cowden disease to 10q22–23. *Nature Genet.* in press. The data obtained will be presented and discussed.

PP-1-16 Apoptosis-Loss and Expression of Death-Related Genes in Breast Carcinomas

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We recently suggested that apoptosis-loss contributes to the metastatic progression of breast cancer by extending cell survival and thus, allowing the acquisition of gene mutations. To study whether the apoptotic death pathway is altered by the expression of proteins such as Bcl-2 or Bcl-x which have an antiapoptotic activity and the interactions with Bax and Bak proteins that can repress their apoptosis-blocking ability, we analyzed the expression of those genes in a serie of 124 T1 (< 2 cm) breast cancer tumors. Indeed, Bcl-2 overexpression was found to be correlated with apoptosis-loss ($p < 0.001$) in those T1 tumors with wild-type p53. In contrast, Bcl-x was not related to the apoptosis-loss in spite of its co-expression with Bcl-2 gene in 53% of tumors. The analysis of the antiapoptotic effect of Bcl-2 overexpression was greatly demonstrated in Bak positive tumors ($p = 0.019$), indicating its direct blocking effect on Bak death promoting activity.

Nonetheless, the overexpression of Bak in breast cancer tumors, was correlated with Bcl-x overexpression which suggests that it might be also an effective mechanism to the apoptotic death regulation.