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 yeilded predominantly diploid cells. Among the clonal aberrations, clones with 15q deletion (q13–q15, q12–q15, q?) 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 regarts 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 occuring 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 ^{125}I-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 μg/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 adhesion 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 ephitelium. 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.

Furthermore, we found that in absence of Bcl-2 overexpression both Bax and/or Bak overexpression (both present in 60% of cases), increased the apoptosis detected in those tumors (p = 0.017 and p = 0.085 respectively).

Our results further stress the role of Bcl-2 overexpression blocking the apoptosis of breast cancer tumors and thus controlling the Bax and Bak death facilitator activities.

PP-1-17 Circulating Tumor Markers (CEA, MCA, CA 15.3, CA 549) in the Diagnosis Breast Cancer Recurrence after Surgery: 5-Year Results

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Concomitant measurement of 4 serum markers (CEA, MCA, CA 15.3 and CA 549) were performed every 3–6 months in 128 breast cancer patients with no evidence of disease after surgery. After a median follow-up of 5 yrs (range 4–5 yrs) 30 pts (23%) relapsed. In 25 of these at least one marker was abnormal (sensitivity: 83%); the 5 pts with normal marker value at the time of relapse had only local recurrence (soft tissue metastases). The sensitivity of CEA and MCA (32% and 47%) was significantly lower than the sensitivity of CA 15.3 (80%) and CA 549 (81%) (p = 0.02). Ninety-nine pts did not relapse: 90 have normal marker values (specificity: 92%). The predictive value of a positive test and of a negative test is 76% and 95%, respectively. The combination of 2 or more markers does not increase the sensitivity (p = 0.5) and the positive predictive value of CA 15.3 or CA 549 alone. The 5-year results confirm that a single marker determination (CA 15.3 or CA 549) is recommended in the follow-up of pts after surgery for breast cancer.

PP-1-18 Plasma c-erbB2 Concentrations and Response to Chemotherapy in Breast Cancer

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The c-erbB2 oncogene is amplified and/or overexpressed in about 25% of breast cancer. The c-erbB2 overexpression has been related to a poor prognosis and a lower response to chemotherapy [1]. Using an enzyme-immunoassay (Triton Diagnostics, Ciba-Corning France) we determined plasma c-erbB2 concentrations in patients with metastatic [2] and inflammatory breast cancers and examined the potential value of plasma c-erbB2 as a predictive indicator. The cut-off value, determined in 30 healthy women between 20 and 80 years, was 27 U/ml. Patients with a c-erbB2 concentration higher than 27 U/ml were considered as c-erbB2 positive (c-erbB2+). 10 out of the 33 metastatic and 9 out of the 25 inflammatory breast cancer patients were c-erbB2+. The response to chemotherapy was not significantly different between c-erbB2+ and c-erbB2– patients with metastatic (4/10 vs 10/23) and inflammatory (6/9 vs 11/16) breast cancer. Plasma c-erbB2 assay has no predictive value in metastatic and inflammatory breast cancer patients.

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[1] Gusterson et al, 1992, J Clin Oncol, 10, 1049–1056.
[2] Révillion et al, 1996, Eur J Cancer, in press.

PP-1-19 Determination of Cytosol ERBB-2 Protein in Primary Breast Cancer

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To determine over-expression of c-erbB-2 gene in breast cancer, ELISA using anti-c-erbB-2 MoAb was performed with cytosol fractions of 139 resected breast cancer specimens from patients with stage I–III B in 1994–1995. Cut-off value was set at 18 ng/mg protein to correlate with gene amplification. The median and mean value of erbB-2 protein were 7.1, 18.7 ng/mg protein, respectively. The positive rate was 18.7%. Positive erbB-2 was associated with histological grade and serum CEA level, but not with tumor size, stage, vessel invasion, nodal status, intraductal component, serum CA15-3 level and PR. There was a week inverse relation in erbB-2 level and ER. The prognostic importance will be evaluated in future.

PP-1-20 Psychosocial Correlates of Oestrogen and Progesterone Receptors in Breast Cancer: Results of Three Consecutive Studies

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Psychosocial correlates of hormone receptor status (assessed by DCC assay) were investigated in 93 consecutive patients attending a radiotherapy service. Life event, coping style, and psychological adjustment self-report scales were completed. The 75 patients with receptor-positive (RP) (oestrogen and/or progesterone) were better adjusted psychologically than the 18 patients with receptor negative (RN) lesions. The results of this first study have been replicated in a sample of 89 consecutive patients hospitalized for breast surgery. The 73 patients with RP were better adjusted psychologically before and after surgery than the 16 patients with RN lesions. The high level of distress found in the first and second study and the high prevalence of psychiatric history in RN group of patients led us to design a third study matching 11 patients with RN lesions with 11 patients with RP lesions for medical and sociodemographic data and comparing the two groups for life events and psychological adjustment. The results of this third study are showing that patients with RN lesions are reporting significantly more long lasting stressful life events before cancer diagnosis. These three studies are indicating that the main psychosocial variables related to RN status is a significant psychological distress related to long lasting stressful events preceding cancer diagnosis. The relationship found here between hormone receptor status and psychosocial variables contributes to the understanding of possible effects mediated by the central nervous system on breast cancer initiation and progression.

PP-1-21 Correlational Study of Microangiographic and Immunohistochemical Techniques for Tumour Vascularisation

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Tumour vascularity is an important predictor of prognosis in breast cancer. We have correlated microvessel density with prognosis in 177 with primary breast cancer. In a smaller prospective series of 21 mastectomy patients we have studied the pattern of neovascularisation and vessel density by the new technique of microangiography and immunohistochemistry (IHC) using monoclonal antibodies to CD34, basic fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) by immunoperoxidase IHC. Microangiograms were done on the gross specimens and there after IHC was done on 4 μm thin sections of tumours. Microangiograms revealed two distinct vascular patterns — an anastamosing pattern (15/21) and a radial pattern (4/21). In 2/21 there was no distinct pattern. Six angiograms were graded 1 (lowest vessel count), 11 graded II and 4 were graded III (highest vessel count). IHC vascular counts correlated with the angiogram grade. Correlation between vascular counts, angiogram grade and angiogenic growth factors will be presented.

PP-1-22 Non-Invasive Measurement of Antioestrogen Activity in the Breast

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Tamoxifen, an antioestrogen with agonist and antagonist properties, is currently being assessed as a long-term chemopreventative agent in patients at high risk of breast cancer. Surrogate markers of Tamoxifen action on the normal breast are needed to assess it's action in individual women. The protein pS2 is stimulated by oestrogen in cancer cell lines in vitro, whilst the breast cyst protein Apolipoprotein D is inhibited by oestrogen also in vitro. Both proteins can be measured in breast secretions. Healthy women (n = 63) and women with breast pain (n = 15) provided breast secretion samples. Sequential samples were collected in women treated for breast pain with Tamoxifen (n = 6) and Zoladex (n = 9) to determine if measurement of these proteins could be used as antioestrogen markers. Apo D and pS2 were measured by radioimmunoassay and total protein by the Bradford method.

Results Premenopausal secretion levels of pS2 ($p < 0.02$) were significantly higher and Apo D significantly lower ($p < 0.03$) than postmenopausal values. Women with breast pain had significantly higher pS2 (median 19.6 vs 8.5 ng/mg protein. $p < 0.04$) and lower Apo D (median 59.9 vs 159.9