respectively ($p<0.001$). Kohen-kappa homogeneity test revealed that breast cancers in each patient differed as regards histological type (MBC $p=0.85$, SBC $p=0.83$) but not as regards histological grade (MBC $p<0.01$, SBC $p'<0.001$). BRCA1 mutation was detected in 12 out of 120 patients (10%). In 8 of them Ins C was revealed. Patients with BRCA1 mutation were younger (43 vs 48 years), more often had MBC (8/12 pts) and had family history of breast cancer (55%). Eleven out of 12 patients with BRCA1 mutation live without any symptoms of the disease. BRCA2 mutation was detected in 1 (0.8%) out of 120 pts and CHEK2 mutation – in 7 (6%) out of 120 pts.

Conclusions: Cancers in two breasts of the same patient differ as regard histological type. Overall survival of bilateral breast cancer is good, but there is a significant difference between SBC and MBS. Patients with BRCA1 and BRCA2 mutation have a good prognosis. Other mutations should be searched, especially in older patients and with SBC.

304 POSTER

Prognostic profiling of node negative untreated breast cancer patients based on outcome: genomic fine-tuning

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Background: Various prognostic molecular signatures for breast cancer patients have been recently published, some of which have been recently validated on independent series, with a good prognostic value on distant recurrences.

However, all existing signatures fail to provide useful information on the type of recurrence expected, which could have significant impact on clinical management. On behalf of the TRANSBIG Network which will be initiating a large prospective trial of the clinical usefulness of genomic profiling, we initiated a study aimed at defining molecular profiles for several subgroups of patients based on their outcome.

Materials and methods: Untreated consecutive node negative breast cancer patients with available tumour samples were selected based on their outcome in two different French cancer centres (Institut Gustave Roussy and Centre René Huguenin): patients who did not relapse (NR)after minimum 10 years of follow-up, patients with a local relapse (LR), 30 patients with distant metastais before 5 years after initial diagnosis (M1), and 23 patients with distant metastasis after 5years of initial diagnosis (M2). Gene expression profiling using the Agilent technology was performed at Institut Gustave Roussy. A specific prognostic gene signature was defined for each sub-group of patients.

Results: A total of 15O patients were included in the present analysis: 63 NR (IGR = 39, CRH = 24), 33 LR (IGR = 14, CRH = 19), 30 M1 (IGR = 17, CRH = 13) and 23 M2 (IGR = 11, CRH = 12).

Discussion: Results of the comparison of molecular profiles of each subgroup and their prognostic value will be presented. This approach should provide some insight into pathways of local and metastatic recurrence and allow more accurate prediction of outcome for node-negative breast cancer patients.

Publication

Breast cancer – basic science, molecular predictive assays, translational research

305 PUBLICATION

Expression pattern of E cadherin in invasive ductal breast carcinoma

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Introduction: E-cadherin (E-CD) is considered to be the most important cell adhesion molecule in mammary gland. Some studies suggest that downregulation of E-CD and subsequent loss of cellular adhesiveness correlate with poor prognosis and metastasis but this is not confirmed by other studies. Many investigations suggest that E-CD protein is not expressed in invasive lobular in comparison with invasive ductal carcinoma (IDC) of the breast. A few papers report that E-CD mediated cell adhesion system can be disrupted by oncoprotein c-erbB-2/HER-2/neu in c-erbB-2-positive breast carcinomas despite ductal or lobular type.

Purpose of study: To evaluate the expression patternt of E-CD and relationship with the status of HER-2/neu in IDC and analyze an association with lymph node positivity.

Methods: We reviewed 91 cases of IDC. All cases were examined in our laboratory for suspicion for c-erbB-2 overexpression. Nottingham histologic grade, immunohistochemical staining for estrogen and progesterone receptors (ER and PR), proliferating cell nuclear antigen (PCNA), E-CD; fluorescense in situ hybridization for HER-2/neu gene amplification; and lymph node positivity were evaluated.

Results: HER-2/neu gene amplification was observed in 60.5% of IDC and positively correlated with higher histological grade and lymph node positivity. Strong positivity for PCNA was observed in 84.2% of IDC and positively correlated with histological grade and HER-2/neu positivity. IDCs were negative for ER in 50%, and PR in 57.8% of cases. ER/PR-negativity was associated with histological grade, HER-2/neu gene amplification and lymph node positivity. E-CD expression was lost in 26.3% cases of IDCs and positively correlated with histological grade, HER-2/neu gene amplification and lymph node positivity.

Conclusion: The loss of E-CD expression can be a feature of some typical invasive ductal carcinomas of the breast. E-CD negativity seems to be associated with higher histological grade, HER-2/neu gene amplification and lymph node positivity suggesting that c-erbB-2 may act as a regulator of E-CD expression in most human breast carcinomas *in vivo*.

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306 PUBLICATION

The immunohistochemical expression of estrogen receptor beta in breast cancer and its correlation with selected clinicopathological parameters and with survival

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Background: The role of estrogen (ER) and progesterone receptors (PgR) in breast cancer is well established. Recently, another type of estrogen receptor, termed ERβ has been discovered. The "classical" ER is now called estrogen receptor α (ERα). While ERα and PgR assays have been routinely used for a number of years, the role of ERβ is still undefined. The aim of this work was to determine the extent of ERα, ERβ and PgR immunohistochemical expression in breast cancer and to determine if the ERβ expression is correlated with selected clinical parameters, biological markers and with survival.

Methods: Formalin-fixed, paraffin embedded breast cancer tissues used in our study came from 110 women who had undergone surgery at our department between 1998–1999. None of the patients had been treated pre-operatively with endocrine therapy. Immunostaining for ERα, ERβ and PgR was performed using monoclonal antibodies against ERα, PgR (DakoCytomation), and against ERβ (CHEMICON). The EnVision detection system was applied. The data were analyzed using a nonparametric Fisher-Freeman-Halton test and log-rang test for disease-free survival (DFS) and overall survival (OS). The statistical significance was considered when $p<0.05$.

Results: 61% of tumors were ERα positive, 64% were PgR positive and 55% were ERβ positive. As many as 14% of ERβ positive tumors had no expression of ERα. In tumors expressing ERβ, the expression of p53 was less common and ERβ positive tumors were of a lower histological grade. There was no correlation between ERβ expression and tumor size and axillary node involvement. Patients with tumors expressing ERβ had better DFS (5 years follow-up), but there was no statistically significant difference in OS.

Conclusions: The expression of ERβ was significant in breast cancer and was also present in a noticeable proportion of ERα negative tumors. Future studies will be required to determine the clinical significance of ERβ in breast cancer.

307 PUBLICATION

The expression of CCR7 in breast cancer tissue and CCL21 in lymph node does not correlate with sentinel node metastasis

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Background: Lymph node metastasis is a major prognostic factor for breast cancer patients. Sentinel node (SN) is defined as the nearest lymph node(s) from primary tumor, however, the factor(s) that can affect on SN metastasis has not been elucidated yet. On the other hand, some types of chemokines have been known to correlate with breast cancer metastasis. Among them, CC chemokine receptor7 (CCR7) is expressed on breast cancer cells, and the CCR7 ligand CCL21 is expressed selectively in lymph nodes. The aim of the present study was to examine the relationship between CCR7 protein expression of primary breast cancer and CCL21 expression of lymph nodes, including SN, and to explore whether CCR7 and/or CCL21 expression in breast cancer patient correlate with SN metastasis.

Materials and methods: Sentinel lymph node dissection (SLND) has been performed among stage I-IIA breast cancer patients during breast operation at Niigata University Hospital since 1999. All these patients underwent axillary lymph node dissection after SLND. Paraffine-embeded sections of primary tumor, SNs and non-SNs were cut postoperatively. Each section of primary tumor was stained with anti-human CCR7 antibody (R&D), and SNs and non-SNs were stained with anti-human CCL21 antibody (R&D). The staining pattern and intensity of primary tumor, SNs and non-SNs were examined, respectively.

Results: Among the patients who underwent SLND, complete sets consisting of primary tumor, SNs and non-SNs were available from13 patients: 5 patients with metastasis positive in both SNs and non-SNs, 5 with only positive in SNs, and 3 with only positive in non-SNs. Furthermore, complete sets from SNs and non-SNs metastasis-negative patients were also examined. The CCR7 protein expression was observed in almost all breast cancer tissues. The CCL21 expression was observed in both SNs and non-SNs, and there was no difference in staining pattern or intensity between SNs and non-SNs, or between metastasis positive and negative nodes. However, the nodes in which metastasis occuppied more than half area, showed extremely decreased expression of CCL21.

Conclusions: These results suggest that there is no difference in CCL21 expression between SNs and non-SNs, and that the CCR7-CCL21 axis will not be a main factor for the formation of sentinel node metastasis.

308 PUBLICATION

Effect of palm tocotrienols on 4T1 mouse mammary cancer cells

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Tocotrienols, isoforms of Vitamin E, are not only known for their antioxidant, lipid-lowering properties and as anti-proliferating agents but also for their inhibitory effect on the growth of human breast cancer cells *in vitro* and *in vivo*. In this study, the effects of Tocotrienol-rich-fractions (TRF) from palm oil and its individual fractions (α-, δ- and γ-tocotrienol) were examined in 4T1 mouse mammary cancer cells. 4T1 cells were cultured and grown in RPMI medium supplemented with different concentrations of tocotrienols. Cell numbers were determined at the end of an incubation period of twelve days. Results showed that TRF and individual fractions of palm tocotrienols inhibited the growth of 4T1 cells *in vitro* at lower concentrations (6–20 μg/μl) compared to tocopherols (>20 μg/μl). δ-tocotrienol was found to be most inhibitory followed by γ-tocotrienol with complete inhibition at 6 and 10 μg/μl respectively.

Levels of apoptosis induced in 4T1 cells before and after treatment with TRF were also determined by flow cytometric analysis of Annexin V staining. There was a significant increase in apoptotic activity in cells after treatment with palm tocotrienols.

Tumourigenesis was examined and compared against control in a BALB/c mice model. The mice were injected with 4T1 cells and were fed palm tocotrienols by oral gavage. There was a lower tumour incidence (37.5%) and higher latency in tocotrienol-supplemented mice, when compared to the control group (87.5%). This study shows that palm tocotrienols have strong inhibitory effects on the growth of 4T1 cells both *in vitro* and *in vivo*.

309 PUBLICATION

Over expression of Bcl-2 protects a highly sensitive human breast cancer cell line against N1, N11-diethylnorspermine-induced apoptosis

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The purpose of the study was to investigate if an over expression of the Bcl-2 gene protects a highly sensitive human breast cancer cell line against spermine depletion. The cells pool of natural polyamines is depleted after treatment with the spermine analogue N^1, N^{11}-diethylnorspermine (DENSPM). This occurs through the down-regulation of polyamine biosynthetic enzyme activities and by up-regulation of the polyamine catabolic enzyme spermindine/spermine N^1-acetyltransferase (SSAT). A unique Swedish human breast cancer cell line, L56Br-C1, is highly sensitive to DENSPM treatment. DENSPM treatment induces mitochondrially-mediated apoptotic cell death in L56Br-C1 cells.

To elucidate the role of interaction between the mitochondria and antiapoptotic proteins, L56Br-C1 was transfected with the Bcl-2 gene to give an over expression of Bcl-2 protein. The cells were treated with 0.1 and 10 μM DENSPM and samples for various analyses were collected after 24 and 48 hours after treatment. Flow cytometry showed a substantial increase of cells in the sub-G_1 peak in the DENSPM-treated control cells, whereas the transfected cells had only a slight increase in sub-G_1 peak. Cells in the sub-G_1 peak is an indication of ongoing cell death. The activation of SSAT is also measured as well as the polyamine levels in order to elucidate the effects of DENSPM on control cells and Bcl-2 over expressing cells. The levels of cytochrome *c*, Bcl-2, Bax, pro-caspase-3 and survivin are investigated by Western blot. In DENSPM-treated control cells, a cleavage of the inactive procaspase-3 into the active caspase-3 should be seen.

Since the cleavage of caspase-3 is an important step in the initiation of the apoptotic cascade, our results may elucidate if DENSPM-treated control cells die by apoptosis whereas increased levels of Bcl-2 may protect the cells against apoptosis. DENSPM is presently in phase II clinical trials for cancer.

310 PUBLICATION

The use of a panel of monoclonal antibodies to enrich circulating breast cancer cells facilitates their detection

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Objective: Metastatic relapse due to early dissemination of tumour cells is associated with poor prognosis for epithelial cancer. The molecular characterization of these single cells or cell clusters that have evaded the tumour is indispensable in order to evaluate their biological behaviour and metastatic potential. In this study we established a sensitive immunomagnetic method to isolate rare cancer cells from peripheral blood based on their expression of epithelial or tumour cell-specific markers.

Methods: Low numbers of cells of breast cancer cell lines, ZR-75–1, MCF-7, HBL-100, were spiked into peripheral blood specimens of healthy volunteers. Enrichment of tumour cells was performed using either pre-coupled HEA and/or ErbB2 microbeads, or a mixture of three monoclonal antibodies against HEA, ErbB2 and EGFR.

Results: The recovery rate of spiked tumour cells correlated with the expression of the corresponding antigens. ZR-75–1 cells high expressing all three genes could be isolated to 60–71%. MCF-7 cells, which hardly express EGFR, showed a significant better recovery by using two specific antibodies in combination (50–68%) than one pre-coupled bead alone (31–42%). HBL-100 cells little expressing HEA could not be isolated with HEA-microbeads and only to 27% in combination with ErbB2 beads – in contrast the use of an antibody cocktail achieved 38%.

Conclusion: As tumour and epithelial specific cell marker antigens are expressed differently in disseminated tumour cells, the immunomagnetic enrichment from peripheral blood is most robust and reliable when using a combination of specific antibodies compared to single antibodies.

311 PUBLICATION

Different cell cycle kinetic effects of N1N11-diethylnorspermine-induced polyamine depletion in four human breast cancer cell lines

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Cells require certain levels of the polyamines – putrescine, spermidine and spermine – to undergo normal cell cycle progression. Depletion of the intracellular polyamine pools results in growth inhibition. Polyamine pools can be depleted in the cell by treatment with polyamine analogues. Polyamine analogues deplete the polyamine pools by inhibiting their biosynthesis and stimulating their catabolism. The analogues cannot take over the normal function of the polyamines in the cell.

In this study, four breast cancer cell lines were treated with the spermine analogue N1N11-diethylnorspermine (DENSPM). The four cell lines (MCF-7, SK-BR-3, HCC 1937 and L56Br) have different genetic aberrations resulting in different basal levels of cell cycle regulatory proteins. Using a bromodeoxyuridine-DNA flow cytometry method, we determined the effect of DENSPM treatment on the rate of G1/S transition, the lengths of the S phase and the G2+M phase. Cell cycle kinetics was affected differently in the four cell lines with SK-BR-3 being least sensitive while L56Br-C1 were most sensitive. In MCF-7, HCC 1937 and L56Br-C1 cells, the rate of G1/S phase transition was decreased and the S phase prolonged after 24 hours of treatment with 10 mM DENSPM. In L56Br-C1 cells, there was also a complete block of the G2+M phase at 24 hours of treatment.

In SK-BR3 cells, the first effect found was on the S phase at 48 hours of DENSPM treatment. In HCC 1937 cells, the G2+M phase was also prolonged at 48 hours of treatment while it still was not affected in MCF-7 cells. L56Br-C1 cells had died by apoptosis at 48 hours of treatment. All these effects on the cell cycle kinetic were correlated to changes in cell cycle regulatory proteins; cyclin D1, cyclin E, cyclin A2, cyclin B1, cdk2, p27, pRb, E2F1, p53 and p21.