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.