patients) were also found with other techniques (2 with ultrasound used in the standard clinical workup in symptomatic patient). Tomosynthesis is promising for lesion detection and lesion characterisation because of reduced superposition. In symptomatic patients the role of tomosynthesis is not yet fully established, but this technique might be useful, especially when used in combination with mammography.

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Examination of sentinel lymph node in breast cancer by the combination of computed tomography lymphography, blue dye method and fluorescence navigation

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Background: The sentinel lymph node (SLN) biopsy technique is established in the treatment of breast cancer. It is considered that the combination of scintigraphic and blue dye method is needed for SLN biopsy. Recently, computed tomography lymphography (CTLG) with iopamidol and fluorescence navigation with indocyanine green (ICG) was developed for detecting SLN in breast cancer.

We examined efficacy of CTLG and fluorescence navigation.

Method: An iopamidol was injected subareolarly in 51 patients with operable breast cancer. Within two minutes after the 15–30 sec gentle massage of the injection sites, 1.25 mm-thick cross-sectional CT images of the breast and axilla were taken. These images were reconstructed into 3D images to identify the location, size and number of the SLN. All the subjects underwent blue dye method SLNB.

SLNB using the fluorescence navigation was performed in the last 15 patients.

Fluorescence images were obtained using an ICG fluorescence high sensitivity near-infrared video camera system (PDE: Hamamatsu Photonics, Hamamatsu, Japan). When ICG was injected subareolarly, subcutaneous lymphatic vessels draining from the areola to the axilla were visible by fluorescence within a few minutes. The SLN was then dissected by CTLG and fluorescence navigation.

A backup axillary dissection was performed on 31 patients. The accuracy of the procedure was evaluated histologically.

Results: A backup axillary dissection was performed on 31 patients, the identification rate was 93.5% and the false-negative rate was 12.5% (1/8 patients). The identification rate in CTLG was 100% afterwards. Subcutaneous lymphatics and SLN were detectable by fluorescence in all patients. The presumptive region of the skin incision was the same as CTLG by the fluorescence navigation for all cases. There was the case that SLN was not stained with by blue dye method.

Conclusion: SLN navigation by ICG fluorescence imaging and CTLG are a promising technique for further clinical exploration.

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Significance of preoperative Fluorodeoxyglucose-PET for detection of axillary lymph node metastasis

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Introduction: The axillary lymph node status is still considered the single most important prognostic indicator in patients who have breast cancer. Clinical examination is generally unreliable for staging the axilla. Lack of conventional imaging techniques to determine the axillary lymph node involvement with acceptable accuracy has been the main reason for axillary lymph node dissection; however, up to 70% of patients who have stage T1 and T2 tumors have negative axillary lymph nodes. The extent, morbidity, and cost of the staging procedure of axillary lymph node dissection are often greater than those of the surgical treatment of the primary tumor. In anatomical based imaging modalities, such as computed tomography, ultrasound, and MRI, the size of a particular lymph node is of crucial importance to determine the tumor involvement. Generally, lymph node enlargement over 1 cm in diameter is the decisive criterion. In contrast, metabolic imaging with FDG-PET is suggested to provide more specific information, based on detecting increased glucose consumption of cancer tissue. This study was undertaken to evaluate the diagnostic accuracy of preoperative positron emission tomography.

Methods: A retrospective review from January of 2007 to December of 2007 was performed in all patients (n = 109) undergoing a preoperative FDG-PET. PET imaging with the radiolabeled glucose analogue (F-18 FDG) was used to visualized the primary breast tumor and metastatic lesions.

Results: In 109 patients, the sensitivity and specificity of PET for detection of axillary lymph node metastasis were 74% and 80%, respectively. Overall accuracy was 79%. In patients (n = 42) who had primary breast tumors larger than 2 cm (>stage pT1), the sensitivity increased of 100%, with corresponding specificity of 82.3%.

Conclusion: FDG-PET cannot replace the axillary dissection, not only because of the limited sensitivity, but also because the number of involved lymph nodes and extranodal extension cannot be determined. But, among patients who have larger tumors, sentinel biopsy can be avoided in those who have positive FDG-PET, in whom complete axillary lymph node dissection should be the primary procedure.

Wednesday, 16 April 2008 12:30–14:30

POSTER SESSION

Tumour biology and immunology

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Breast cancer-derived factors stimulate ERK-mediated survival of osteoclasts and limit the effectiveness of bisphosphonates in treatment of bone metastases

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Introduction: Bone metastasis is a common complication of advanced breast cancer, which causes distressing symptoms, including pathological fractures and bone pain. Osteoclasts are critical mediators of bone osteolysis induced by breast cancer. Moreover, osteoclasts further drive tumor growth by releasing IGF and TGF-β stored in the bone matrix. Osteoclast-directed therapy is thus an ideal strategy to treat breast cancer metastases to bone. Bisphosphonates are the only available anti-osteoclastic agents used in metastases but their effect is limited in the metastatic setting relative to other osteolytic conditions. We sought the explanation of osteoclast resistance to bisphosphonates and the potential avenues to inhibit osteoclasts in breast cancer metastatic bones.

Materials and Methods: Osteoclasts formed from RAW 264.7 cells under effect of the physiological osteoclastogenic mediator RANKL (50ng/ml) were treated with Alendronate for 48 hours in the presence or absence of conditioned media from the MDA-MB-231 breast cancer cell line culture. The numbers of multinucleated osteoclasts stained positive for tartrate-resistant acid phosphatase were assessed in parallel samples, and the levels of ERK1/2 phosphorylation were determined by immunoblotting. Some cultures were treated with the ERK inhibitor PD98059.

Results: Raw 264 cells develop mature osteoclasts after 5 days of treatment with RANKL. Exposure of mature osteoclasts to 10% MDA-MB-231 conditioned media significantly increased the cell count at day 7 compared to cultures that were continuously treated with RANKL treatment, indicating that soluble mediators released from breast cancer cells support osteoclast survival in vitro. ERK pathway has been previously shown to play a critical role ion osteoclast survival. We have found that exposure to soluble factors from breast cancer cells strongly induced phosphorylation of ERK1/2 in mature osteoclasts. Alendronate treatment of RANKL-treated mature osteoclasts resulted in significant and dose-dependent decrease in the osteoclasts count. In contrast, in the presence of breast cancer-derived factors, mature osteoclasts failed to respond to alendronate in the concentration range from 10^{-8} to 10^{-4} M, demonstrating that osteoclast exposed to breast cancer soluble factors are resistant to the apoptotic effect of alendronate. Inhibition of ERK pathway using PD98059 (10 μM) before treatment with Alendronate (10^{-4} M) partially restored the responsiveness of mature osteoclast to Alendronate.

Conclusion: Osteoclasts exposed to cancer-derived factor demonstrate improved survival likely counteracting pro-apoptotic effects of alendronate. Identification of the mechanism of alendronate resistance in breast metastases may pave the way to more effective breast cancer bone metastases.

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CD44+/CD24−/low cells derived from long-term cultured human breast carcinosarcoma

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The CD44+/CD24−/low cells have been recently identified as breast cancer-initiating cells. They retain tumorigenicity in vivo and display stem cell-like properties. We have obtained CD44+/CD24−/low (>90%) cells from carcinosarcoma using mammosphere culture method which was previously

used to isolate breast stem/progenitor cells. While most of the primary cells were adherent and terminally differentiated within a few passages in vitro, the mammosphere could be maintained as floating spheres for more than 50 passages in vitro. While most of the primary cells were adherent and terminally differentiated within a few passages in vitro. We cultured only the floating cells, which could be maintained for more than 50 passages in vitro. The floating cells were stained positive for fibronectin while negative for epithelial markers CK14 and CK18. Interestingly, nestin and tuj 1 were also expressed in these floating cells suggesting that they may posess multipotency to differentiate into other cell types. In differentiating medium containing FBS, floating cells became adherent and their CD44 expression levels were significantly decreased. This might imply that CD44 may be responsible for maintaining self-renewal of the mammospheres. We have also found that both mammospheres and derivative adherent cells could efficiently form tumors in NOD/SCID mouse. Taken together, our results suggest that our mammospheres could be a suitable in vitro model to study breast cancer-initiating cells.

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Differential control of alveolar and ductal development in grafts of rat mammary clonogenic epithelial cells

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Purpose: It is possible to reproduce the original mammary shape and touch by grafting patient's own mammary cells using the same mold produced for their marry shapes from patients who have an operation of mastectomy if the cultivation of the mammary gland is possible. This study attempts to cultivate mammary glands using clonogenic epithelial cells in the mammary gland of rats.

Methods: Fluorescein isothiocyanate-peanut agglutinin (PNA-FITC) and phycoerythrin-anti-Thy-1.1 monoclonal antibody (Thy-1.1-PE) were applied to selectively differentiate these clonogenic cells. From the results of the analysis of the mammary epithelial cell of 7−8 weeks old F344 rats using a flow cytometry, it was possible to sort four different cell groups, such as a cell group (B−) that represents negative for these two markers, a cell group (PNA+) that represents positive in the PNA-FITC, a cell group (Thy-1.1+) that shows positive in the Thy-1.1-PE, and a cell group (B+) that represents positive for these two markers.

Results: A single PNA+ cell was sorted from the donor in order to investigate the implantability in vivo using a flow cytometry. Then, it was injected into the interscapular and lumbar fat pad in a hyperprolactin state of MtT F4-grafted recipient F344 rats. After three weeks from the injection, it was verified that alveolar unit structures were generated at a 2.93% of the implanted site. In addition, it was verified that ductal unit structures were generated at a 8.33% of the implanted site after injecting the multicellular structures that was produced from the PNA+ cell, which was cultivated in the Matrigel over one week, into the fat pad of hyperprolactinglucocorticoid-deficient F344 rats.

Conclusion: Therefore, it was evident that the PNA+ cell group among mammary epithelial cells of rats possesses many of the characteristics of multipotent clonogenic stem-like cells and the potential to be differentiated into various types of cells according to the environmental control of hormones in vivo.

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Docetaxel-induced apoptosis of human umbilical vein endothelial cells (HUVECs) is mediated by the activation of MAPK and modulation in Bcl-2 family proteins

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Background: Among many chemotherapeutic agents, docetaxel is known as one of the most potent inducer of apotosis. And also docetaxel have anti-angiogenic effect by inducing apoptosis in endothelial cells. We investigated the intracellular events that occurs during the process of apoptosis in human umbilical vein endothelial cells (HUVECs) by docetaxel.

Materials and methods: HUVECs were grown as a monolayer in sterile endothelial growth medium (EGM-2). After 72 hrs, the cells were treated with docetaxel at the concentrations ranging from 0.05 nM to 100 nM for 24–48 hrs. After the 24 hrs of docetaxel treatment, the cells were treated for 1 hr with the Fluorescent Inhibitor of Caspases (FLICA) Apoptosis Detection Kit specific for activity of the poly-caspases to observe early apoptosis. After the 48 hrs of docetaxel treatment, cell viability was determined using cell counting kit 8. Cell lysates were prepared and Western blot analysis was performed with specific primary Abs [ERK1/2, phospho-ERK1/2, p38, phospho-p38, Bcl-2 and Bax].

Results: The IC50 of HUVECs treated with docetaxel was 1.0 nM and docetaxel inhibited the proliferation of HUVECs in a dose-dependent manner. Caspase activity was increased with the dose dependent manner of docetaxel. 0.1 nM of docetaxel caused time dependent phosphorylation of ERK1/2, and maximum activity was seen between 24−48 hrs. Exposure to docetaxel caused the concentration dependent phosphorylation of ERK1/2 and p38 after 48 hrs. During this early process of apoptosis, up-regulation of pro-apoptotic Bax protein and down-regulation of anti-apoptotic Bcl-2 protein were observed in a dose dependent manner ranging from 0.01 nM to 10 nM after 48 hrs of exposure to docetaxel. Consequently, Bcl-2/Bax ratio in HUVEC was decreased by docetaxel in dose dependent manner, maximal at 48 hrs. Then we evaluated the change in Bax protein level after treatment with inhibitors of ERK-1,2 and p38. Remarkable reduction in Bax protein level was observed upon ERK1/2 inhibitor treatment in a dose dependent manner, but there was no change in Bax protein level upon p38 inhibitor treatment.

Conclusions: It seems that docetaxel induces transient activation of ERK1/2 and is responsible for dose dependent up-regulation of proapoptotic Bax proteins, whereas p38 plays a role in apoptosis independent with the modulation of Bcl-2 family protein.

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Dolichyl phosphate and polyprenol could inhibit P-glycoprotein in human MCF-7 breast cancer cells

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Background: Multidrug resistance (MDR) in cancer cells during chemotherapeutic course restricts the possibilities of cytostatic application. The investigations reveals that MDR correlates with MDR1 gene expression and accumulation of P-glycoprotein (Pgp) in plasma membrane. The resent results are in favour of the idea that glycoprotein synthesis in malignant tissues is limited by Dolichyl Phosphate (DolP). The aim of the present study is to investigate the effect of polyprenol (PP) which provides a DolP substitute in regulation of N-glycosylation on MCF-7 breast cancer MDR.

Methods: Breast cancer cell lines, MCF-7 and MCF-7 cells with induced resistance to Doxorubicin (MCF-7/ADR) were used. Polyprenols (PP) concentration in the culture medium made up 10^{-3}–10^{-8} M. MDR1 expression was assessed by an immunohistochemical technique. DolP and Pgp fractions were analysed by HPLC methods.

Results: Polyprenol in concentration 10^{-3}–10^{-4} M induced apoptosis in MCF-7 cells within 3−4 hours. It is confirmed that plasmatic membrans of MCF-7 cells contain 5.6−6.4% of Pgp (the total protein amount) as a resistance marker. Resistant MCF-7/ADR cells differ from sensitive ones MCF-7 in Pgp content by 10−12 times. The study showed 8.5-fold DolP decrease in MCF-7/ADR cells. The investigations demonstrate that the situation can be changed by treatment with DolP and PP. The DolP concentration in MCF-7/ADR cells was returned to the normal level. It is established that DolP in the concentration 10^{-6} M aid 7–9-fold reducing Pgp in membranes of MCF-7/ADR cells. The MCF-7/ADR cells cultivation in medium with polyprenol proceeded to give lowered Pgp content in membranes no over 0.4−0.6%, which amount was consistent with the level of Pgp in MCF-7 cells.

Conclusions: These results indicate that noncontrollable accumulation of Pgp, after MDR1 expression in MCF-7/ADR cells can be overcomed using stimulation with dolichyl phosphate substitution. Polyprenol is a promising new agent which usage can open up possibilities to tackling the problem of MDR in breast cancer chemotherapy.

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Immunological detection, characterization and prognostic value of circulating tumor cells in patients with advanced breast cancer

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Background: Immunologic detection and characterization of circulating tumor cells (CTC) in peripheral blood and bone marrow of patients with advanced breast cancer (ABC).

Materials and Methods: Sixty five patients with ABC (38 pts were newly diagnosed, 27 pts had relapse of BC) were examined for CTC before treatment (6−8 courses of chemotherapy or 3−6 months of endocrine therapy), twenty one patients – after the treatment. For the multi parameter flow cytometry detection and characterization of CTC in peripheral blood of all patients we used fluorochrome-labelled monoclonal antibodies (mAbs) BerEp4 (BD, USA) and HEA125, antigenic peculiarities of tumor cells being detected by mAbs to HLA-DR and CD95. Forty two patients were examined