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

for tumor cells in bone marrow aspirate immunocytologically with antibody to cytokeratin KL-1 before treatment and 11 pts – after the treatment. Time to progression (TTP) and overall survival (OS) were analyzed to find out whether the presence of CTC influenced prognosis in ABC.

Results: At least one per million CTC are found in 41.3% of patients with ABC before systemic treatment and in 45.5% of patients – after treatment. The CTC level is not related to site or number of metastatic tumor, not to receptor status of the primary breast tumor. The CTC are found more frequently in menopausal than in premenopausal woman (47.8% vs 26.7%, $p = 0.044$). Patients with infiltrative lobular BC present with CTC significantly more frequently than those with infiltrative ductal BC (69.2% vs 31%, $p = 0.02$). Bone marrow involvement has no influence on CTC detection frequency. CTC-positivity has no effect on OS or TTP of patients with ABC. Analysis of CTC levels before and after systemic treatment demonstrated that patients with diminution of CTC number after treatment had significantly longer time to disease progression than those with increased CTC levels after treatment (14 vs 8 months, $p = 0.0127$). HLA-DR expression on CTC was found in 82% of patients (mean percentage of HLA-DR+CTC was 31.6%), CD95 was expressed in 33% of patients (mean percentage of CD95+CTC was 14.8%).

Conclusion: Increase in CTC levels after specific anticancer treatment may be a new useful, objective measure of response and an informative unfavorable prognostic factor in patients with ABC.

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Gene expression analysis of the insulin- and estrogen signalling system and their influence on clinical parameters of breast cancer patients

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Background: Obesity is thought to increase breast cancer risk both through high activity of estrogen metabolizing enzymes and by associated high insulin and glucose serum levels directly impacting on proliferation and apoptosis of breast cancer cells. In the present study, we investigated co-expression of genes of the insulin/IGF axis and the estrogen signaling system in correlation to clinical parameters in collected breast cancer specimen.

Materials and Methods: Breast cancer tissue and fasting serum was collected from 26 female patients. After microdissection of the frozen samples, RNA was isolated and expression of genes of the estrogen- and insulin/IGF signaling was measured by real time RT PCR. Fasting insulin, glucose and C-peptide as well as estradiol serum level were analysed by ELISA. Insulin resistance was calculated by the HOMA method.

Results: Postmenopausal women older than 70 years showed significant higher expression of estrogen receptor (ER) α as well as steroid sulfatase (STS) and were more likely insulin resistant than premenopausal women younger than 50 years. A strong significant association between vascular/lymphovascular invasion (L1, V1) and BMI as well as insulin resistance could be identified. Both, ERα and STS expression were significantly associated with expression of insulin receptor, IGFR1 and IGFBP4 but not IGFR2. Higher expression of IGFR1 was associated with a better histological grading, whereas higher expression of IGFR2 correlated with lymph node negativity.

Conclusion: In conclusion, the observed co-expression of components of the insulin/IGF signaling with ERα and steroid sulfatase supports the hypothesis that a close cross talk between both pathways is present in breast cancer cells. The observed correlation of insulin resistance with vascular invasion encourages further studies on larger case numbers to further examine the relevance of this association in the clinical situation.

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CAV1 and CAV2 are associated with breast cancer basal-like and triple negative immunophenotype

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Background: Caveolin-1 (CAV1) and caveolin-2 (CAV2) are the principal structural proteins of caveolae, sphingolipid and cholesterol-rich invaginations of the plasma membrane involved in vesicular trafficking and signal transduction. Over the recent years there has been controversy about their role in breast cancer and their suitability as markers of basal-like phenotype.

The aims of this study were threefold: (1) To assess the prevalence of CAV1 and CAV2 in a well-characterized series of invasive breast carcinoma using high-throughput tissue microarrays (TMAs) and immunohistochemistry. (2) To determine whether CAV1 and CAV2 could be used as diagnostic marker to identify basal-like phenotype or the triple negative (TN) subtype of invasive breast cancers (3) finally to identify if CAV1 and CAV2 have any prognostic effect on the patient outcome in invasive breast cancer.

Material and Methods: CAV1 and CAV2 protein expressions were assessed on a tissue microarray containing 880 unselected invasive breast cancer cases, by means of immunohistochemistry.

Results: CAV1 and CAV2 expression was observed in 13.4% and 5.9% of all breast cancer, respectively. Their high expression was strongly associated with high histological grade, lack of steroid hormone receptor positivity (ER and PR), and expression of basal markers (basal cytokeratines, P63, P-cadherin). Furthermore there was a significant association between CAV1 and CAV2 expression and basal-like phenotype. On univariate analysis only CAV2 had a prognostic effect on breast cancer-specific survival; however, this was not independent from other traditional markers on multivariate analysis.

Conclusion: Our results demonstrate that both CAV1 and CAV2 are associated with basal-like phenotype. Further studies are warranted to determine whether they play a role in basal-like/triple negative breast cancer development or are just surrogate markers for this subgroup.

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Relation of intratumoral B-Cells and response to neoadjuvant chemotherapy

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Introduction: Tumor-infiltrating immune cells have contradictory effects on tumor growth and angiogenesis. In early stage cancer, intratumoral T Cells attenuate tumor progression, as shown for node negative colorectal cancer, and are a better predictor of patient survival than the standard histopathological staging. Conversely, in later stages, cancer cells recruit macrophages to release mitogenic factors (e.g. EGF), to remodel the extracellular matrix and to facilitate angiogenesis. However, hardly any data exists on the influence of the immune response of breast cancer patients receiving neoadjuvant chemotherapy in particular in view of antibody based chemotherapy. In this study we analyzed the expression of IGHM as a marker of the presence of B cells in samples of tumor tissue of patients treated with anthracycline and trastuzumab in relation to therapy response.

Patients and methods: Breast cancer patients ($\geqslant$cT2, N0/N1, M0) received neoadjuvant chemotherapy of 4 cycles of epirubicin and cyclophosphamide followed by 4 cycles of paclitaxel (PREPARE trial). Her-2/neu positive tumors were treated with trastuzumab in a three weekly dose regimen (TECHNO trial). Tumor cell content and histology were centrally determined from a HE stained reference slide. RNA was isolated from tissue sections of 10μm thickness by an automated system based on magnetic beads (Siemens Medical Solutions Diagnostics). IGHM was analyzed in an initial group of 56 patients (33 TECHNO/23 PREPARE) by TaqManPCR. IGHM expression was correlated with ESR1, Her-2/neu, TOPO2A and VEGF expression and to histopathological findings in excised tumors.

Results: 10 of the 56 patients included in the preliminary analysis showed a full remission at histopathological evaluation. There was a significant difference of IGHM-RNA expression for those patients with histopathologically complete response to systemic therapy and those with no or partial response (Mann-Whitney-U, $p = 0.047$). Patients with a complete response showed higher levels of IGHM-RNA indicating presence of B-cells. Interestingly, IGHM expression correlated with increased proliferation and vascularization as determined by TOPO2A (Spearman $r = 0.41$; $p = 0.0008$) and VEGFC (Spearman $r = 0.43$; $p = 0.0005$) RNA expression, but not with ESR1 and Her-2/neu status.

Conclusion: In advanced breast cancer, the presence of B cells correlates with proliferation and angiogenetic activitiy. Also, the presence of B cells is increased in tumors that respond to chemotherapy. These data support the hypothesis that the immune response has an influence on the behavior of breast cancer tumors. The analysis of tumor-infiltrating immune cells may therefore be a valuable prognostic tool in breast cancers patients.