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.

180 Poster

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

H. Kwak[1], Y. Bae[1], K. Choo[2], S. Lee[1], J. Lee[1], K. Park[3]. *[1]Pusan National University Hospital, Surgery, Busan, Korea; [2]Pusan National University Hospital, Radiology, Busan, Korea; [3]Pusan National University Hospital, Nursing, Busan, Korea*

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.

181 Poster

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

J. Kim[1], J. Lee[1], H. Kim[1], E. Chang[1]. *[1]Chungnam National University Hospital, general surgery, Daejeon, South Korea*

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.

182 Poster

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

I. Kuznecovs[1], K. Jegina[1], S. Kuznecovs[1]. *[1]Preventive Medicine Research Institute, Cancer Research Laboratory, Riga, Latvia*

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.

183 Poster

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

O. Bzhadug[1], N. Tupitsyn[2], S. Tjulandin[3], L. Grivtsova[2]. *[1]Cancer Research Center, Clinical pharmacology department Immunology of hemopoesis, Moscow, Russian Federation; [2]Cancer Research Center, Immunology of hemopoesis, Moscow, Russian Federation; [3]Cancer Research Center, Clinical pharmacology department, Moscow, Russian Federation*

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