Correlation of these 30 cases with ER status and HER2/neu expression revealed that 24/30 ($p < 0.05$) demonstrated ER positive status and 14/30 HER2/neu positive expression. Methylation of RAR2b was observed in 22/52 pts. Correlation of these 22 cases with ER status and HER2/neu expression revealed that 18/22 ($p < 0.05$) demonstrated ER positive status and 10/22 HER2/neu positive expression. Remarkable observation is that out of the total number ($n = 20$) of HER2/neu positive pts, 14 presented alongside with methylation of RASSF 1A gene ($p < 0.05$) while 10 presented methylation of RAR2b gene. Both genes were methylated in 16/52 pts. Notable observation is also that out of the total number of pts with both genes methylated 10 demonstrated ER positive status and 6 HER2/neu positive expression.

Conclusions: RASSF 1A and RAR2b are commonly methylated in primary BC. Methylation of either RASSF 1A or RAR2b genes was not correlated with HER2/neu positive status. In contrast, we demonstrated that both genes were significantly methylated in ER positive tumors. The last observation may be of significance in the evaluation of targeted therapy in ER positive pts which do not respond to endocrine therapy. The small number of pts does not allow us to confirm the exact role of RASSF1A and/or RAR2b genes methylation in primary BC yet. Larger studies are required in order to assess if these epigenetic alterations point out new BC markers, which could be helpful in prognosis and potential biological therapeutic strategies.

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Estrogen receptor positive advanced breast cancers with high cytokine content and Treg were associated to high percentage of complete pathological response under primary docetaxel and epirubicin or trastuzumab chemotherapy

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Background: An emerging hypothesis suggests that cytokine could play an important role in cancer as potential modulators of angiogenesis and tumor infiltration leucocytes (TIL).

Material and Methods: Multiplexed flow cytometry technology was used to measure the expression of cytokines in TIL: TCD8+IL17, TCD4+IL17 and CD4+ CD25+ (Treg), and IFN-γ production by CD3 stimulation in 30 advanced breast cancer (IIB to IIIB) under neoadjuvant chemotherapy scheduled three cycles of docetaxel 75 mg/m^2 and epirubicin 50 mg/m^2 or docetaxel and trastuzumab 6 mg/kg (as HER-2 positive) (q3w).

Results: Cytokines expressed in TIL breast carcinoma were correlated to estrogen receptor and progesterone receptor status (IL17 and IFN-γ). Cytokines were correlated with age at cancer diagnosis, tumor size, histological type, lymph node status, and IL-17, CD8+ IFN-γ and Treg (CD4+CD25+, Fox P3, CTLA4 and GITR) were more abundant in low-grade tumors than in high-grade tumors (HER-2 positive). In addition, IFN-γ produced by CD8+ stimulated by CD3 was expressed to a greater degree in HER2-positive than in HER2-negative patients. Pathological response was evaluated in excised specimens by surgery after three cycles of chemotherapy. Twenty one of 30 patients were estrogen positive receptors (70%). Ten of them were associated to high levels of cytokines (47.6%). In this group we have four complete pathological responses (40%) of the total six in all groups (20%) (p 0.001).

Conclusions: Our study demonstrates a high pathological response rate with primary docetaxel and epirubicin/Trastuzumab chemotherapy in estrogen receptor positive locally advanced breast cancer with cytokines over expressed (IL17 and IFN-γ) and Treg. It could be correlated with inflammatory cell component, which could account for the best prognosis of these tumors and maybe future prognostic and predictive factors in advanced breast cancer therapy.

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The effect of insulin analogues on telomerase catalytic subunit expression in breast cancer cells

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Background: Insulin analogues which are modified versions of human insulin are widely used to control blood sugar in people with type 1 and type 2 diabetes. It has been suggested that modifying the insulin molecule can increase its mitogenic potency and increase the risk of cancer. Recently it has been shown that some of the insulin analogues have significantly higher proliferative effect on breast cancer cells. In this study the effect of regular insulin, glargine, aspart and NPH (neutral protamine hagedorn) was investigated on the expression of the gene of telomerase catalytic subunit (hTERT). Telomerase is a ribonucleoprotein enzyme which is responsible for lengthening of chromosome ends. This enzyme which is not active in most human somatic cells is activated in cancer cells and thus allows continuous cellular proliferation and cancer development.

Material and Methods: Human breast adenocarcinoma cell line (MCF-7) was cultured in RPMI-1640 medium supplemented with 10%fetal bovine serum (FBS) and incubated with regular insulin and its analogues including NPH, glargin and Aspart. To examine the expression of hTERT, total cellular RNA was extracted, cDNA was synthesized and semi-quantitative real-time PCR was performed.

Results: None of the insulin analogues significantly increased the expression of the catalytic subunit of telomerase 48 hours after treatment of MCF-7 cells.

Conclusion: Our results showed that insulin analogues did not have a significant effect on telomerase expression in breast cancer.

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The sensitivity of hormone-resistant breast cancer cells to doxorubicin: the role of NF-kappa B signaling

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Background: The resistance of breast cancers to growth stimulating estrogen action may provokes the paradoxical tumor sensitization to estrogen apoptotic action. 17beta-estradiol suppress NF-kB, demonstrating the possible NF-kB involvement in the estrogen apoptotic action. The present work was performed to study the influence of estrogens on the sensitivity of the resistant breast tumors to cytostatic drugs, and to evaluate the role of NF-kB signaling in the regulation of the survival of the resistant breast cancer cells.

Material and Methods: Resistant MCF-7/LS subline was developed by long-term cultivation of the parental cell line MCF-7 in steroid-free medium. The transcriptional activity of NF-kB and estrogen receptor was determined using luciferase reporter gene assay. The knock-down of NF-kB was performed by the cell transfection with small interfering RNA. The apoptosis level was evaluated by flow cytometry using staining with propidium iodide.

Results: 17beta-estradiol enhances the apoptotic action of doxorubicin in the resistant MCF-7/LS breast cancer cells. The proapoptotic estrogen action is mediated by NF-kB suppression when NF-kB knock-down sensitizes the resistant cells to both estrogen and doxorubicin.

Conclusions: Estrogen-induced NF-kB suppression in the resistant breast cancers results in a imbalance between pro- and anti-apoptotic pathways and cell sensitization to anti-tumor drugs. Additional inhibition of NF-kB by siRNA increases the apoptotic action of estrogen and doxorubicin, demonstrating that NF-kB may be considered as a potential target in the therapy of the resistant breast cancers.

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A correlation between breast cancer recurrence and circulatory tumour cells detected by cytokeratin 20 in the peripheral blood and bone marrow

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Background: Most patients present stage I or II breast cancer and at least up to 30−40% of these patients will develop recurrent disease. These patients are considered as having disseminated circulatory tumor cells at the time of local treatment. Cytokeratin (CK) 20 is expressed in a majority of breast cancer, but expressed in normal tissues or benign breast disease. The aim of study is to evaluate a correlation between the outcome of breast cancer and circulatory tumor cells detecting by cytokeratin 20. And then, we found the role of CK amplification as prognostic predictor.

Material and Methods: Between Jan 1999 and Aug 2003, the sample of blood and bone marrow was obtained from breast cancer who undertaken optimal surgical treatment at Korea University Hospital. We analysis 117 paired sample of blood and bone marrow using Real time PCR. A case who was revealed metastasis was excluded. A period of mean follow-up was 55 months.

Result: Each expression of CK 20 in the blood and bone marrow were shown in 31 (26.5%) cases and 48 (41%) cases, respectively. The expression of CK 20 in both was found in 19 (16.2%) cases. A significant difference of disease free survival between expression of CK20 in both and absent or only one sample was founded ($P = 0.02$).

Conclusion: Over-expression of CK 20 in the both of blood and bone marrow is a useful predictor for the recurrence of breast cancer.