Results: The genomic status of regions located on chromosomes 2p22.2, 3p23 and 8q21−24 and the number of segmental alterations were defined in the training set to classify tumors into low or high-risk groups. In the validation set, this CGH classifier produced a highly significant odds ratio of 10.39 (95% CI: 3.75–28.78, $p = 6.63 \times 10^{-6}$, Wald test) in univariate analysis with a sensitivity of 66%, a specificity of 84% and an accuracy rate of 78%. The 5-year metastasis-free survival analysis showed a highly significant difference between the two predicted groups (Hazard Ratio = 5.7, $p = 1.82 \times 10^{-7}$, log-rank test). Together with estrogen receptor and grade, this CGH classifier provided significant prognostic information in multivariate analysis.

Conclusions: In addition to classical parameters, this DNA signature may constitute an accurate tool to identify patients with T1T2N0 luminal tumors, who may benefit from adjuvant treatments.

EG, GP, AV-S, XS-G, BA and OD contributed equally.

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HER-2/neu expression in T1 to T3 breast cancer with extracapsular extension of axillary lymph node metastasis

S. Maksimovic[1], B. Jakovljevic[2]. [1]*General Hospital Sveti Vracevi, General and Oncologyc Surgery, Bijeljina, Bosnia-Herzegovina;* [2]*Clinic Center Banja Luka, Clinic of Oncology, Banja Luka, Bosnia-Herzegovina*

Background: Studies on the association of HER-2/neu with the axillary lymph node metastasis are controversial. Amplification of the protein product of the HER-2/neu oncogene in primary breast cancer specimens is associated with an adverse prognosis.

Methods: From January 2000 to January 2009, 504 breast cancer patients operated in General hospital “Sveti Vracevi” in Bijeljina. We selected 253 (50.2%) patients with breast cancer who had metastases to axillary lymph nodes.

Results: Extracapsular extension (ECM) was found in 103 (40.7%). The patients were identified and divided into two groups: patients in the HER-2 positive group (38 patients) and HER-2 negative group (65 patients). In the HER-2 positive group ECM was seen in 62.5% patients compared with 37.4% in the HER-2 negative group (P = 0.059). 41 patients (39.8%) were identified with three or less lymph nodes involved, 30 patients (29.1%) patients four to six, 20 patients (19.4%) seven to nine, and 11 patients (10.6%) ten or more nodes, respectively. Total number of lymph nodes showing ECM were also significantly more in the HER-2 positive group (48 of 81, 59.25%) *vs.* (13 of 60, 21.66%) in the HER-2 negative group ($P < 0.001$). With a median follow-up of 96 months factors with independent prognostic value for disease-free survival by multivariate analysis included HER-2/neu overexpression with extracapsular extension ($P < 0.005$), pN category ($P < 0.01$), presence of lymphovascular invasion (LVI; $P < 0.005$), and ECM ($P < 0.001$). An independent negative prognostic effect on overall survival was observed for HER-2/neu overexpression with extracapsular extension ($P < 0.05$), pN category ($P < 0.05$), and presence of LVI ($P < 0.005$) and ECM ($P < 0.001$).

Conclusions: In patients whose tumors expressed HER-2/neu who had positive lymph nodes and extracapsular extension prognosis was significantly worse compared with those who were HER-2/neu negative and lymph node positive with extracapsular extension. These findings have led to the conclusion that HER-2/neu overexpression is associated with a more aggressive subtype of cancer.

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TNF superfamily gene polymorphism as prognostic factor in early breast cancer

J.H. Jung[1], S.M. Hwangbo[1], H.G. Jung[1], H.Y. Park[1], Y.S. Chae[2], J.Y. Park[3]. [1]*Kyungpook National University Hospital, Surgery, Daegu, Korea;* [2]*Kyungpook National University Hospital, Hemato-oncology, Daegu, Korea;* [3]*Kyungpook National University Hospital, Pathology, Daegu, Korea*

Purpose: Since apoptosis may play a role in the prognosis of breast cancer, the present study analyzed the polymorphisms of apoptosis-related genes and their impact on the survival of 240 patients with early invasive ductal breast cancer.

Methods: The genomic DNA was extracted from paraffin-embedded tumor-free tissue or blood, and 12 single nucleotide polymorphisms (SNPs) of 11 apoptosis-related genes in the apoptosis pathway determined using a Sequenom MassARRAY system.

Results: During the median follow-up of 53.4 (range, 2.9–205.9) months, 37 relapses and 22 deaths occurred. Among the target polymorphisms, the tumor necrosis factor superfamily member 10 gene polymorphism (TNFSF10 rs1131532) in a recessive model of the T allele and prostaglandin-endoperoxide synthase 2 gene polymorphism (PTGS2 rs5275) in a dominant model of the C allele were associated with survival in a log-rank test. The TT genotype of TNFSF10 (rs1131532) was also significantly correlated with a lower disease-free, distant disease-free, and overall survival in a multivariate analysis (HR = 3.304, 4.757, and 6.459; $P = 0.002$, 0.001, and 0.009, respectively), while PTGS2 rs5275 was only associated with a higher distant disease-free survival (HR = 0.302; $P = 0.041$). No clinicopathologic difference was observed according to the genotypes of these two polymorphisms.

Conclusion: The TNFSF10 (rs1131532) polymorphism was identified as a possible prognostic factor of survival in patients with operated invasive breast cancer.

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Reference gene selection to quantify urokinase plasminogen activator in breast cancer

R. Esmaeili[1], K. Majidzadeh-A[1], N. Abdoli[1], M. Habibi[1], A. Bahrami[1]. [1]*ICBC, Genetics Research Group, Tehran, Iran*

Background: Cancer biomarker research has been improved using real-time PCR. Normalization of target gene expression to a control gene is a common way to quantify gene expression changes. Some housekeeping genes have been used widely for quantification. Several studies show that the expression of some housekeeping genes alters in breast cancer tissues; hence they are not suitable for gene expression analysis. Therefore finding stable genes will help to investigate gene expression properly.

Method: In the study 7 common housekeeping genes in breast cancer tissues were selected and their stability was examined in order to normalize expression of Urokinase Plasminogen Activator (UPA) which is important in metastasis. Reference genes were analyzed with Real-time PCR are as follow: HPRT1, GAPDH, RPLP0, β actin, TFRC, β2M and GUSB. RNA from Breast cancer tissue along with their normal adjacent tissues was extracted using RNX-plus (Cinnagen, Iran). cDNA synthesis was done with reverse transcription kit (Primer design Ltd, UK). Primers and probes were design using GeneRunner version 3.05 and primer Express software version 3. Real-time PCR was carried out using precision 2X mastermix (Primer design Ltd, UK) and fluorescent detection was performed using Applied Biosystems 7500 System. The data was analyzed using geNorm soft ware which uses pairwise comparison approach in order to find the most stable genes.

Result: The most stable genes were RPLP0 and HPRT1 while GAPDH was the least stable gene.

Conclusion: In this study HPRT1 and RPLP0 were the best house-keeping genes for UPA normalization in breast cancer. Different studies suggest other genes as two of them will be explained. Mc Neill et al, suggest MRPL19 and PPIA as the most stable and RPLP0 as the least stable gene, but Lyng et al, recommend TBP, RPLP0 and PUM1 for normalization. As different studies have special condition and they use some of housekeeping genes in their studies, various genes may be found as the best reference for normalization, some of them are common in various researches. Testing more housekeeping genes will help to find the best genes, but different treatment and situation in research may change the expression of housekeeping and it is better to check the stability of controls based on experiment design to find the proper genes.

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Correlation between CpG methylation profile of RASSF 1A and RAR2b genes with estrogen receptor (ER) and HER2/neu status in primary breast cancer (BC)

K. Desiris[1], S. Voyatzi[2], A. Kiziridou[3], D. Sahpazidou[2], E. Stergiou[4], S. Theodosiadi[5], P. Stravoravdi[2], G. Simbilidis[1]. [1]*Theagenio Cancer Hospital, 3rd Surgical, Thessaloniki, Greece;* [2]*Theagenio Cancer Hospital, Research, Thessaloniki, Greece;* [3]*Theagenio Cancer Hospital, Pathology, Thessaloniki, Greece;* [4]*Theagenio Cancer Hospital, 2nd Oncology, Thessaloniki, Greece;* [5]*Theagenio Cancer Hospital, 2nd Surgical, Thessaloniki, Greece*

Background: ER positive BCs are considered prognostically more favorable than ER negative, whereas HER 2/neu positive BCs are associated with worse prognosis. We examined the methylation status in the CpG islands of two major breast tumor-related genes RASSF1A and RAR2b in relation to ER and HER2/neu status in primary BCs.

Materials and Methods: Patients with BC (n = 52), randomly selected, were included. Genomic DNA was extracted from archive formalin-fixed paraffin-embedded tumor tissues. DNA methylation was determined by chemical modification of DNA and subsequent double “hot start” Methylation-Specific PCR (MSP), followed by detection on agarose gel. A polyclonal antibody against HER2/neu was used for immunohistochemistry. Results were classified according to the Herceptest criteria: (negative (0/1+), weakly positive (2+) and positive (3+).

Results: Methylation of at least one of the genes was observed in 36/52 pts. Methylation of RASSF1A gene was observed in 30/52 pts.

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

H. Marana[1], D. Tiezzi[1], L.G.O. Brito[1], V.F. Schiavon[1], W.S. Clagnan[1], J.M. Andrade[1]. [1]*School of Medicine of Ribeirao Preto, Gynecology, Ribeirao Preto SP, Brazil*

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

M. Nourbakhsh[1], M. Razzaghy Azar[2]. [1]*Iran University of Medical Sciences, Clinical Biochemistry, Tehran, Iran;* [2]*Iran University of Medical Sciences, Institute of Endocrinology and Metabolism, Tehran, Iran*

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

A. Scherbakov[1], Y. Lobanova[2], O. Andreeva[2], V. Shatskaya[2], M. Krasil'nikov[2]. [1]*Russian N.N.Blokhin Cancer Research Centre, Laboratory of Clinical Biochemistry, Moscow, Russian Federation;* [2]*Russian N.N.Blokhin Cancer Research Centre, Laboratory of Molecular Endocrinology, Moscow, Russian Federation*

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

S. Woo[1], J.W. Bae[1], E.S. Lee[1], J.B. Lee[1], G.S. Son[1], H.Y. Kim[1], C.H. Kim[2]. [1]*Korea University Guro Hospital, General Surgery, Seoul, Korea;* [2]*Korea University Guro Hospital, Pathology, Seoul, Korea*

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.