Conclusion: These studies provide strong evidence that instability in the D-loop region of mtDNA may be involved in breast malignancy. We suggested that mtDNA mutations may play a role in breast cancer development in Romanian patients but their role in the mechanism of carcinogenesis remains to be solved. The use of mtDNA copy number may have a diagnostic value but further studies on a larger cohort of patients are necessary in order to validate it as a potential biomarker for early detection of breast carcinoma.

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265   Poster

Dual Color in Situ Hybridization and Mutational Analysis of Triple Negative Breast Cancer with EGFR Protein Overexpression

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Background: It has been well known that triple negative breast cancer (TNBC) is a heterogeneous tumor and highly express epidermal growth factor receptor (EGFR). Although TNBC with EGFR protein overexpression has been extensively studied, systematic studies of EGFR protein overexpression and gene amplification are rare. We studied correlation of EGFR protein overexpression with EGFR gene amplification, EGFR activating mutation, and expression of components of Akt pathway.

Materials and Methods: Tumor tissues were obtained from 84 patients with TNBC. We analyzed the status of EGFR, phosphorylated Akt (pAkt), phosphorylated mammalian target of rapamycin (p-mTOR), and other markers using immunohistochemistry (IHC). We also analyzed EGFR gene and chromosome7 copy numbers by DISH. Tumors with $\geqslant$15 copies in $\geqslant$10% of cells or EGFR gene cluster ($\geqslant$4 spots) in $\geqslant$10% of cells or EGFR signal/chromosome7 signal ratio $\geqslant$2 were interpreted as positive for gene amplification in this analysis. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) samples. Analysis of EGFR gene activating mutations were performed for these samples using the peptide nucleic acid (PNA)-clamp smart amplification process version 2 (SmartAmp2).

Results: Characteristic histopathological types of TNBCs with EGFR overexpression are typical medullary carcinoma, apocrine carcinoma, small cell carcinoma, metaplastic carcinomas, and adenoid cystic carcinoma. EGFR protein overexpression was found for 28/84 (33.3%), but EGFR gene amplification was not detected. Chromosome7 polysomy expression (positive score $\geqslant$4%) and high polysomy expression ($\geqslant$4 copies in $\geqslant$ 40% of cells) were found in 46/84 (54.7%) and 6/84 (7.1%) samples, respectively. There were significant correlations between EGFR protein overexpression and each of chromosome7 polysomy and high polysomy expression. But we could not find any relation between EGFR protein overexpression and each of pAkt and p-mTOR and the other clinicopathlogical factors. Although we were able to analyze EGFR gene activating mutations in 55 (65.4%) of 84 FFPE samples, we found no evidence of EGFR gene activating mutations in these 55 samples.

Conclusions: Several clinical trials have been performed to test the role of anti-EGFR directed therapy for TNBC with EGFR protein overexpression. The present study indicates that chromosome7 polysomy expression might be a candidate biomarker for selection of targeted therapy.

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MiRNA-218 Regulation of Human D-glucuronyl C5-epimerase Expression in Breast and Prostate Cancer

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Human D-glucuronyl C5 epimerase (GLCE) is one of the key enzymes of the biosynthesis of heparan sulfate proteoglycans (HSPG) involved in cell-cell and cell-matrix interaction and signaling. GLCE catalyses epimerization of D-glucuronyc acid into L-iduronic acid residues in the heparan sulfate polysaccharide chains which gives the flexibility to the HS chains and facilitates their interaction with numerous extracellular ligands including growth factors. We have recently demonstrated that GLCE expression is significantly decreased both in breast and prostate tumours and its ectopic expression inhibits cancer cell proliferation *in vitro* and tumour growth *in vivo* suggesting a potential tumour-suppressor function of GLCE. However the mechanism of the down-regulation of GLCE in cancer is still unclear.

In the study, molecular mechanisms of GLCE inactivation were investigated with a special attention on the possible miRNA involvement in GLCE regulation in cancer. Design of the study included the measurement of GLCE and miRNA-218 expression in the same breast and prostate tumours by Real Time RT-PCR analysis, Western blot and TaqMan Small RNA Assay. Correlation of GLCE and miRNA expression levels was calculated with OriginPro 8 software. To investigate the effect of miRNA-218 on GLCE expression *in vitro*, miRNA-218 and anti-miRNA-218 were transfected into breast and prostate cancer cells (MCF7, PC3, DU145) and GLCE expression level was determined by Real Time RT-PCR and Western blot.

It was shown that promoter methylation is not involved in the regulation of GLCE expression in cancer cells while a chromatin structure affects it both directly and indirectly (through the activation of some positive GLCE regulators). miRNA-218 is able to regulate GLCE protein level in breast and prostate cancer cells *in vitro* with GLCE mRNA level being unaffected. However, no significant correlation between GLCE and miRNA-218 in breast and prostate tumours was shown.

The obtained data revealed a complex regulation of GLCE expression in cancer cells and breast and prostate tumours. Different molecular mechanisms contribute to the regulation including chromatin structure and miRNA-218 which affects GLCE expression *in vitro* but not *in vivo*. Possibly the miRNA-218 influence on GLCE expression *in vivo* is more complicated due to an involvement of some other miRNA-218-regulated proteins.

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Correlation Between Expression of NF-kB and ODC with Other Molecular Markers in Tumors of Patients with Breast Cancer

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Background: The aim of research is to study NF-kB (p50/p65), ornithine decarboxylase (ODC), c-erbB2, ER, PR, Ki-67, p53 expression in breast tumors and analyze correlation between these markers.

Methods: We selected 69 patients with breast cancer before treating with radio- or chemotherapy. The I stage was diagnosed for 5, II − 44, III − 8, IV − 2 patients, 10 patients had 'X' stage at that moment. All tumors belong to invasive ductal carcinoma with different Grade (G). Expression of molecular markers was determined by immunohistochemistry on paraffin-embedded tissue sections.

Results: The correlation between G and ER (r = −0.995, p < 0.05); G and Ki-67 (r = 0.995, p < 0.05); ER and p53 expression (r = −0.987, p = 0.052) was defined.

We grouped the patients with high levels of p50 and p65; high p50 and low p65; low levels of subunits. The correlation between expression of NF-kB and hormone receptors (ER: r = −0.999, p < 0.01; PR: r = −0.999, p < 0.05) was evaluated. Then we divided the patients with high level of p50 into two subgroups: first − with high ODC level, and second − with low ODC level. It was shown the decrease of ER expression (r = 0.991, p < 0.01) and increase of p53 accumulation (r = 0.997, p < 0.01) in this direction: p65−/p50− − p65−/p50+/ODC+ − p65−/p50+/ODC− − p65+/p50+.

Also, NF-kB, ODC, Ki-67 and p53 expression in different molecular types of breast tumors was analyzed. Breast tumors are divided on Luminal (ER+/c-erbB2−), HER2 (ER−/c-erbB2+), Luminal-HER2 hybrid (ER+/c-erbB2+), and basal-like (ER−/c-erbB2−) types. HER2 type had the highest expression of p65 (r = 0.878, p = 0.061), p50 (r = 0.908, p < 0.05), p53 (r = 0.970, p < 0.05) and basal-like type had the greatest level of proliferation (Ki-67: r = 0.989, p < 0.01).

Conclusion: The correlation between G and molecular markers (ER and Ki-67) was defined. ER and p53 levels were found to be changed in dependence of NF-kB/ODC expression profile. Also, correlation between NF-kB, Ki-67 and p53 expression and tumor type was shown. So, our findings are the reason for further research in this field, because the results of such investigations may be useful for prognosis of disease flowing.

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Analysis of Large Genomic Rearrangements of BRCA1/2 Genes in Korean Breast Cancer Patients

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Background: The BRCA1 and BRCA2 genes are associated with inherited susceptibility to breast and ovarian cancer. Most of disease-causing

mutations in BRCA1/2 are point mutations and small insertion/deletions, but recently increasing number of large genomic rearrangements in BRCA genes have been reported in different populations with various prevalence. However, little is known about the prevalence and types of genomic rearrangements of BRCA genes in the Korean population. In this study, we have analyzed for the presence of BRCA1/2 large genomic rearrangements in Korean breast cancer patients.

Methods: Multiplex ligation-dependent probe amplification (MLPA) was used to screen BRCA1/2 large genomic rearrangements in Korean breast cancer patients (249 for BRCA1 and 215 for BRCA2) at a priori risk of BRCA1/2 mutations due to known risk factors. The patients have been comprehensively analyzed for germline mutation in the entire regions of the BRCA1 and BRCA2 genes, using using a combination of fluorescent-conformation sensitive capillary electrophoresis (F-CSCE) and direct sequencing, and were found negative. Positive MLPA result was confirmed and located by long-range PCR and sequencing.

Results: We identified one large deletion in BRCA1, deleting exon 13−15, in one patient with family history of breast cancer. Breakpoints of this deletion are novel. However, we could not find any large deletion in BRCA2.

Conclusion: Our results suggest that the large genomic rearrangements in BRCA1/2 genes are not a major cause for increased breast cancer susceptibility in Korean population.

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Ki67 in Breast Cancer Patients and Its Correlation with Clinico Pathological Factors

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Background: Ki-67 is a nuclear protein universally expressed in all proliferative tissues. Large number of studies have confirmed the potential use of Ki-67 as a prognostic indicator and in predicting response to treatment in early breast cancer. However, due to variation in analytical practice, measurement of Ki-67 is still not recommended for management of early breast cancer patients. The aim is to study our initial results of measurement of Ki67 in breast cancer patients and correlate its significance with known prognostic factors.

Table 1. Clinico pathologic indicators and the Ki-67 index in primary breast cancer patients

Ki-67 index	<20%	20−50%	>50%
Mean tumour size, in cm	2.7	3.4	3.0
Mean age, in years	60.2	59.9	58.1
Breast Cancer Subtypes			
Luminal A (%)	80.9	10.7	8.4
Luminal B (%)	64.3	21.4	14.3
HER 2 type (%)	55.6	22.2	22.2
Triple Negative (%)	44.4	0	55.6
Total (%)	74.1	12.1	13.8
Positive Nodes (%)			
0	67.2	20.9	11.9
1−3	30.8	38.5	30.8
⩾ 4	40.0	30.0	30.0
Nuclear Grade (%)			
1	100	0	0
2	84.8	13.0	2.2
3	43.5	26.1	30.4
Estrogen receptor (%)			
Positive	78.2	12.9	8.9
Negative	44.4	16.7	38.9
Progesterone receptor (%)			
Positive	83.1	14.5	2.4
Negative	50.0	11.1	38.9
HER2 (%)			
1+	75	10	15
2+	74.6	13.4	11.9
3+	54.5	27.3	18.9

Materials and Methods: The Ki67 index was measured in all patients with early breast cancer in our breast unit over a period of six months.

Prognostic factors such as Estrogen Receptor, Progesterone Receptor and HER-2 Receptors were simultaneously measured. Ki 67 index was categorised based on median value of 20% and analysed in accordance with breast cancer subtypes.

Results: Ki67 index was measured in 119 patients from February 2011 to July 2011. Age ranges from 29 to 89 with the mean tumour diameter of 2.8 cm (range from 0.6 to 11 cm). The median value of Ki-67 index was 20% and of the subtypes were Luminal A 12.5%, Luminal B 20%, HER 2 type 30% and Triple Negative tumours was 70%. Table 1 shows the relationship between the different groups of Ki67 index (<20%, 20−50% and >50%) and the clinic-pathological indicators of primary breast cancer.

Conclusion: The Ki-67 index ranged from 1−98% and the median was 20%, similar to other studies. The median Ki-67 values were different among subtypes, being low for the good prognostic Luminal A type tumours and high for the triple negative tumours. A higher Ki-67 index correlated with negative ER/PR, higher nuclear grade, younger age, larger tumour and positive HER 2. These data suggest that patients with a higher Ki-67 index have a poorer prognosis. It is therefore important to measure Ki-67 index and to be considered in the treatment and follow up of breast cancer patients.

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The NF-kappa B Signalling Pathway and the Response to Doxorubicin in Hormone-resistant Breast Cancer Cells

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Background: Oestrogen is an intrinsic regulator of the breast cancer growth and progression. Many breast cancers are initially sensitive to estrogen and antiestrogen treatment. The acquired tolerance of breast cancers to growth stimulating estrogen action may provokes the paradoxical tumor sensitization to estrogen apoptotic action. The phenomenon of the cell sensitization to estrogen-induced apoptosis has been demonstrated for the breast cancer cells undergoing long-term estrogen withdrawal or antiestrogen treatment. NF-kB is a transcriptional factor that controls apoptosis and cell responses to stress. Earlier studies have shown that estradiol suppress NF-kB, demonstrating the possible NF-kB involvement in the estrogen apoptotic action. The goal of this work is to study the influence of estrogens on the sensitivity of the resistant breast tumors to doxorubicin, and to evaluate the role of NF-kB signaling in the regulation of the survival of the resistant breast cancer cells.

Material and Methods: 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 apoptosis level was evaluated by flow cytometry using staining with propidium iodide.

Results: It has been demonstrated that 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: Thus 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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Quantitative Analysis of RORalpha mRNA Expression in Human Breast Cancer

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Objectives: The retinoic acid receptor-related orphan receptor (ROR) α is a member of the steroid/thyroid hormone nuclear receptor superfamily, which plays an important role on growth and differentiation of many organs by regulating transcription of target genes. We previously reported that RORα directly activated aromatase expression in breast cancer cells through a newly-identified ROR response element located within the promoter region of the aromatase gene. According to previous studies including ours, RORα mRNA is expressed in breast cancer cell lines, such as MCF7. However,