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

H. Nakajima[1], Y. Ishikawa[2], Y. Mitani[3], Y. Ohno[4], M. Furuya[1], J. Horiguchi[5], T. Sano[1], T. Oyama[1]. *[1]Gunma University Graduate School of Medicine, Department of Diagnostic Pathology, Maebashi Gunma, Japan; [2]Gunma University Graduate School of Medicine, Departments of Diagnostic Pathology and Thoracic and Visceral Organ Surgery, Maebashi Gunma, Japan; [3]The Omics Science Center RIKEN Yokohama Institute and K.K. DNAFORM, Yokohama Kanagawa, Japan; [4]Tone Chuou Hospital, Department of pathology, Numata Gunma, Japan; [5]Gunma University Graduate School of Medicine, Department of Thoracic and Visceral Organ Surgery, Maebashi Gunma, Japan*

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

L. Mostovich[1], T. Prudnikova[1], A. Kondratov[2], V. Kashuba[2], I. Ernberg[3], E. Zabarovsky[4], E. Grigorieva[1]. *[1]Institute of Molecular Biology and Biophysics, Laboratory of the Molecular Mechanisms of the Carcinogenesis, Novosibirsk, Russian Federation; [2]Institute of Molecular Biology and Genetics, Molecular Oncogenetics Laboratory, Kiev, Ukraine; [3]Karolinska Institute, Department of Microbiology Tumor and Cell Biology, Stockholm, Sweden; [4]Linkoping University, Institute of Clinical and Experimental Medicine, Linkoping, Sweden*

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

D.O. Shapochka[1], S.P. Zaletok[1], M.I. Gnidyuk[2]. *[1]R.E.Kavetsky IEPOR, Department of Tumor Growth Biochemistry, Kyiv, Ukraine; [2]Ivano-Frankivsk Regional Oncology Dispensary, Department of Pathology, Ivano-Frankivsk, Ukraine*

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

B.H. Son[1], S.H. Ahn[1], J.W. Lee[1], J.H. Yu[1], H.J. Kim[1], B.S. Ko[1], J.Y. Cho[1], J.W. Lee[1], B.K. Ku[1], D.Y. Cho[2]. *[1]Asan Medical Center, Department of Surgery, Seoul, Korea; [2]LabGenomics, LabGenomics Clinical Research Institute, Seoul, Korea*

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