amplification, translocation and other damages during the carcinogenesis. Usually, expression of miRNAs miR-16, miR-122, miR-125a/b, miR-31, miR-143, miR-145 and miR-205 is down-regulated in breast cancer cells whereas expression of miRNAs miR-20b, miR-21, miR-155, miR-19, miR-148, miR-210 and miR-221 is up-regulated. This investigation aims to identify in what way these shifts in miRNA expression pattern contribute to the differences in receptor phenotype of breast cancer cells.

Material and Methods: miRNA targets within gene transcripts were predicted *in silico* using TargetScan software.

Results: miRNAs miR-122, miR-145, miR-148, miR-181, miR-204, miR-18a/b, miR-19, miR-22, miR-221/222 can target transcript of gene ESR1 encoding estrogen receptor alpha (ER). Transcript of gene *PGR* encoding progesterone receptor (PR) carries miR-23a/b, miR-26a/b, miR-135 and miR-181 binding sites. miRNAs miR-125a/b, miR-193, miR-331/331−3p can suppress gene encoding ErbB2 receptor. miR-19, miR-143, miR-145, miR-148, miR-205 silence gene encoding ErbB3 receptor. Transcript of gene encoding ErbB4 receptor carries miR-19, miR-23a/b, miR-125a/b, miR-135, miR-145, miR-193a/b, miR-205, miR-221/222 binding sites. Disregulation of above miRNAs is essential for tumor growth, because downregulated miRNAs silence expression genes of proliferative components E2F1, E2F2, E2F3, RASA1, RASA2, CDK6 as well as gene of main antiapoptotic factor Bcl-2. At the same time, up-regulated miRNAs silence genes encoding cell cycle inhibitors p27 and p57, TGF-β receptor, tumor suppressor pTEN, proapoptotic factors Bak1, FAS as well as genes encoding key elements of cell differentiation network.

Conclusions: Transcripts of genes encoding ER, PR as well as ErbB2, ErbB3 and ErbB4 receptors carry the binding sites for both downregulated and upregulated miRNAs. Therefore, expression or silencing of these genes is a result of balance between shifts in miRNA expression, which are individual for each case of tumor transformation. Different sets of shifts in miRNA expression pattern can cause reactivation of cell oncogenes and antiapoptotic genes as well as repression of proapoptotic, antimetastatic genes and genes of cell cycle inhibitors. Consequently, receptor phenotypes of breast cancer cells can mirror distinct modes of abnormal epigenetic activation of the signal pathways that leads to the tumor growth.

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Overexpression of PGRMC1 ñ a Potential Mechanism for Increased Breast Cancer Risk During Combined Treatment with Estrogen and Norethisterone

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Background: Epidemiological studies suggest that the addition of progestin to estrogen therapy negatively influences breast cancer development. In a recent study from Finland increased breast cancer risk has been observed especially under a combined estrogen (E2)/norethisterone (NET) treatment. Up to now no increased proliferation rate has been detected *in vitro* under this combination. The present study investigates the effects of an E2/NET combination on the proliferation of MCF-7 breast cancer cells overexpressing the progesterone receptor membrane component 1 (PGRMC1). In addition this combination was tested in a mouse transplantation model.

Materials and Methods: MCF-7 cells were stably transfected with a PGRMC1 expression plasmid (MCF-7/PGRMC1−3HA). E2 in concentrations of 10^{-12} and 10^{-10} M was combined with 10^{-7} M NET in a sequential regimen. Proliferation was determined by MTT assay. In transplantation experiments nude mice were injected with 17-beta-E2 pellets (0.72 mg/60 day release) on both flanks 24 h before inoculation of tumor cells. Tumor cells were injected subcutaneously into each flank. After approx. 8 days animals were injected with NET pellets (10 mg/60 day release) or with control pellets. Tumor volumes were recorded twice per week.

Results: Sequential combination of E2 and NET increases the proliferation rate of PGRMC1 overexpressing MCF-7 cells by a factor of 2−3. When the concentration of E2 is reduced the proliferation rate decreases by approx. 50%. Tumors growing from MCF7/PGRMC1 cells in the recipient nude mice are more sensitive to E2. Developed tumors are approx. 4-fold larger compared to transplanted MCF-7 cells, transfected with an empty vector control. NET almost doubles the tumor volume of the MCF-7/PGRMC1 cells compared to animals treated with control pellets.

Conclusions: For the first time we demonstrate in cell experiments and in an animal model that by combining E2 and NET the proliferation of breast cancer cells overexpressing PGRMC1 is increased. In a next step breast tissue or epithelial cells from nipple aspirates from women should be investigated for increased expression of PGRMC1 and women's risk to develop breast cancer after treatment with E2 and NET should be evaluated.

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Cytochrome P450 2D6 Gene Copy Number as an Indicator for Tamoxifen Resistance in Breast Cancer

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Background: Tamoxifen is one of the most effective adjuvant breast cancer therapies available worldwide.

The rate of metabolism of this medicine is mainly determined by the amount of cytochrome p450 2D6 **(CYP2D6)** enzyme expressed in the liver, which is highly variable due to its extensive genetic polymorphisms and copy number variation. So *copy number variation* maybe one of the most important mechanisms of resistance to Tamoxifen. Since there is limited information about CYP2D6 in resistant patients, we aimed to determine copy number of this gene in Tamoxifen resistant Iranian breast cancer patients.

Materials and Methods: *Samples*: The samples of two sensitive and resistant groups were obtained from Iranian Center for Breast cancer Bio-Bank (ICBC-BB). DNA extraction was done using phenol chloroform method and the extracted DNA concentration was quantified using spectrophotometery.

Copy number analysis: Establishment of standard curves for copy number determination was done by cloning of CYP2D6 fragment as the gene of interest and albumin gene as a copy number control in TA cloning vector. PCR Primers were designed using primer express V.3.0 software. Real-time PCR was performed using the ABI 7500 system apparatus. Amplification reactions (20 ul) were carried out in triplicate with 40 ng of template DNA, SYBR Green Master Mix buffer (PrimerDesign Ltd, UK) and 300 nM of each primer. Each sample was run triplicate with 4 fold serial dilutions in the same plate. Samples with standard deviation greater than 0.5 from the mean threshold cycle of the triplicates were excluded from the analysis. Copy number calculation was done using applied biosystems SDS software ver2.0.

Result: Primer efficiency for both CYP2D6 and Albumin was about 100%. The copy number range was 0.4 to 3 and no significant difference was seen between resistance and sensitive groups in this phase of the study.

Conclusion: Although no significant difference was detected between two groups in this phase of the study, it does not mean that copy number variation play no role in resistant group in the samples. Further analysis including genotyping and multivariate analysis considering other factors for tamoxifen resistance and also increasing sample size must be done in order to decide about CYP2D6 status in tamoxifen response in Iranian samples.

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Changes in Estrogen Receptor (ER), Progesterone Receptor (PgR) and HER2/neu Status During Primary Systemic Chemotherapy in Breast Cancer

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Background: The influence of primary systemic chemotherapy (PST) in breast cancer on expression of biological marker is still unclear. In this study, we investigated whether there were any differences in estrogen receptor (ER), progesterone receptor (PgR) and HER2/neu status between core needle biopsies before PST and surgical specimens after PST.

Patients and Methods: One hundred patients with invasive ductal carcinoma who have received PST (PST group) at our hospital were included in this study. The immunohistochemical (IHC) analysis was performed on both core needle biopsies before PST and surgical resection specimens after PST. We also evaluated the expression of ER, PgR and HER2/neu using core needle biopsies and surgical specimens in sixty nine patients without PST (control group).

Results: ER discordance was 4.0% in the PST group, and 2.9% in the control group. PgR discordance was 10% in the PST group, and 7.2% in the control group. HER2/neu discordance was 9% in the PST group, and 2.9% in the control group. HER2/neu expression changed to positive in three cases, and changed to negative in six cases after PST. There were no significant differences in the change of biological marker pattern between the PST and the control group. Discordance of HER2/neu expression tended to be high in the PST group, compared to the control group.

Conclusion: Discordance of biological markers was observed before and after PST. To select optimum adjuvant therapy, HER2/neu status as