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

well as ER and PgR status should be re-evaluated on surgical specimens after PST.

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Comparison of HER-2 and Hormone Receptor Expression in Primary Breast Cancers and Metastases

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Background: Recent retrospective reviews suggest that the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor type 2 (HER2) receptor may differ between the primary and recurrence or distant metastases. In these reports, the rate of discordance for ER, PR and HER2 status ranged between 10 and 30%.

Methods: 42 patients who had tissue samples for both primary and metastatic lesions were eligible for our stud. ER, PR, and HER2 status were determined by immunohistochemistry (IHC) and/or FISH.

Result: The sites of biopsied recurrent/metastatic lesions are regional soft tissue (21.4%), lymph nodes (30.1%), lung (26.1%), bone (16.6%), brain (4.8%), and ovary (2.4%).

Discordance for ER was 11% (n = 5). Among these, 7.1% (n = 3) patients had ER-positive primary tumor but ER-negative metastasis and 4.8% (n = 2) had ER-negative primary but ER-positive metastasis. Discordance for ER was 19% (n = 8). Among these, 14.3% (n = 6) had PR-positive primary but PR-negative metastasis and 4.8% (n = 2) had PR-negative primary and PR-positive metastasis. HER-2 status was known in both primary tumor and metastasis in 34 patients. Among these patients, 15.9% (n = 2) had discordant results. Among these discordant cases, two had negative primaries and positive metastasis and no cases had positive primaries and negative metastasis.

We analyzed the discordance in receptor status between primary and recurrent lesion, by subtypes. 21 patients were defined as luminal type (ER and/or PR+, HER2−), 3 patients were defined as Luminal HER2 type (ER and/or PR+, HER2+), 12 patients were defined as HER2 type (ER−, PR−, HER2+), and 6 patients were defined as Triple negative (TN) type (ER−, PR−, HER2−). Discordance rate in Luminal, Luminal HER2, HER2, and TN was 48%, 0%, 8%, and 16%. There was no significant difference in the rate of discordance according to subtypes.

Conclusions: In the management of recurrent or metastatic breast cancer, we may consider tissue sampling of the metastatic lesions, and identify changes in ER, PR or HER2 status, which could lead to more appropriate therapy.

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DPAGT1 Regulation with Polyprenol in MCF-7 Breast Cancer Cells: Possible Therapeutic Approach to E-cadherin Loss Prevention

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Background: Dysregulation of DPAGT1 (Dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transferase) causes disturbances in E-cadherin expression. In breast carcinomas, loss of E-cadherin correlates with high tumour grade and risk of metastasis. The resent results are in favour of the idea that N-glycosylation in cancer cells is limited by Dolichyl Phosphate Cycle (DPC) intermediates. The aim of the present study is to investigate the effect of Polyprenol (PP), which provides a Dolichol Phosphate (DolP) substitute on regulation of E-cadherin expression in MCF-7 breast cancer cells.

Materials and Methods: Breast cancer cell lines, MCF-7 and MCF-7/ADR were used. PP concentration in the culture medium made up 10^{-2}–10^{-6}. Immunohistochemical and Western blotting methods were used to detect the changes in the expression levels of E-cadherin and DPAGT1 expression. Intermediates of DPC fractions were analysed by HPLC method.

Results: Overexpression of DPAGT1 was 4-fold higher in MCF-7 and 7-fold higher detected in MCF-7/ADR cells than in human mammary epithelial cells (HMEC). Resistant MCF-7/ADR cells differ from sensitive ones MCF-7 in E-cadherin content lost by 3−4 times. It was caused by dolichol-chain shortening and abberant N-glycosylation of E-cadherin in DPC. The study showed 8.5-fold DPC intermediates decrease in MCF-7/ADR cells and 3.6-fold DPC intermediates decrease in MCF-7 cells. Treatment of MCF-7 cells with PP resulted in downregulation of DPAGT1. It is established that PP in the concentration 10^{-4} M could overcome DPAGT1 overexpression which leads to regulation of E-cadherin N-glycosylation.

Conclusions: N-glycosylation is one of the regulators of E-cadherin tumor suppressive activity by affecting the stability of AJs and the assembly of TJs. The findings indicate that DPAGT1 overexpression in MCF-7 and extensive overexpression in MCF-7/ADR can be overcomed by PP, which provides a DolP substitute for DPAGT1 normal expression, N-glycosylation and E-cadherin loss prevention. Polyprenol could be a promising agent for metastasis control in breast cancer.

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Breast Cancer with PIK3Ca Mutations Associated with a Favorable Prognosis in Patients Treated with Tamoxifen Alone

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Background: PI3K/AKT pathway plays a critical role in the tumorigenesis and aggressiveness of human breast cancer. Mutations of *PIK3CA* gene that encodes the PI3K catalytic subunit have been found in approximately 30% of breast cancer. Mutations occur predominately in two 'hot spots' in exon 9 and 20, encoding the helical domain and kinase domain, respectively and constitutively activate downstream signals. Cross-talk between the estrogen receptor and PI3K/Akt pathways are thought to be resistant to hormonal therapy. However some previous studies showed good prognosis in ER positive breast cancer patients with *PIK3CA* mutation. This may be a result of heterogeneous treatment populations in ER/*PIK3CA* mutation positive patients. To clarify this controversy, we sought to examine interaction between *PIK3CA* mutation and breast cancer outcomes in patients who are treated with adjuvant tamoxifen alone.

Material and Methods: Frozen samples from 133 consecutive patients treated with tamoxifen alone as an adjuvant therapy were analyzed for *PIK3CA* mutations using DNA direct sequencing in exon 9 and exon20.

Results: We identified 45 (34.1%) mutations of 133 tumor samples. Of the 45 mutations, 18 (13.6%) and 27 (20.5%) were observed in exon 9 and exon 20, respectively. *PIK3CA* mutation status was not significantly associated with clinicopathologic features such as tumor size, lymph node status, or grade. Patients with *PIK3CA* mutations did not have a worse prognosis compared with those without mutations, but it is not statistically significant due to small number of events.

Conclusions: In our findings, breast cancer with *PIK3CA* mutations associated with a favorable prognosis in patients treated with tamoxifen alone. This may help stratify patients likely to benefit endocrine therapy alone. Further studies are needed to confirm this association.

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Relation Between CpG Methylation 14−3-3-sigma and Nodal Positive Status in Breast Cancers

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Background: 14−3-3 sigma is induced in response to DNA damage, and causes cells to arrest in G(2). Hypermethylation of CpG islands located in the promoter regions of tumor suppressor genes is now firmly established as an important mechanism for gene inactivation. Our objective is to study the relation of 14−3-3 sigma gene promoter hypermethylation and nodal status in sporadic breast cancer.

Material and Methods: This is a prospective study we quantified methylation levels of promoter 14−3-3σ gene in 107 women with breast cancer and 108 control subjects by Real Time QMS-PCR SYBR green and analyzed association with prognostics factor in breast cancer.

Results: Median age was 58 years (32−88); 69% were postmenopausal women. Nodal involvement N0; 63%, N1; 30%, N2; 7%), tumor size (T1; 58%, T2; 35%, T3; 4%, T4; 4%) and grade G1; 20%, G2; 37%, G3; 30%). The methylation of 14−3-3σ were 60% of sporadic breast cancer patients and were 34% of normal breast ($p = 0.0047$). The methylation of 14−3-3σ gene in serum was markedly related with T3−4 stage ($p < 0.05$), nodal positive status ($p < 0.05$) and poor outcome. With a median follow up 6 years we saw more probability of developing distance metastasis in patients with methylation 14−3-3σ ($p > 0.05$).

Conclusions: Hypermethylation of the 14−3-3σ a promoter is an early and frequent event in breast neoplastic transformation, leading to the suggestion that silencing of 14−3-3σ may be an important event in tumor progression and particularly in breast carcinogenesis. Therefore, it is possible that loss of σ expression contributes to malignant transformation by impairing the G_2 cell cycle checkpoint function, thus allowing an accumulation of genetic defects. Perhaps in the detection of CpG methylation of 14−3-3σ may be used for diagnostic and prognostic purposes.