These results suggest that TAM induces CYP3A4 and MDR1 gene expression through SXR, which reduces TAM concentration in breast cancer cells. Thus, we propose that the expression of SXR in breast cancer cells could be a potential risk factor, which induces local TAM resistance.

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Expression of cyclooxygenase-2 in breast carcinogenesis and its relation to Her-2/neu and p53 protein expression in invasive ductal carcinoma

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Although cyclooxygenase-2(COX-2) is overexpressed in various malignant tumors including breast cancers, little is known about the contribution of COX-2 in breast carcinogenesis. Recent studies suggest a possible role of HER-2/neu and p53 gene in controlling COX-2 expression. The purpose of this study was to evaluate COX-2 expression in the successive steps of breast carcinogenesis and to determine its correlation with HER-2/neu and p53 expression in invasive ductal carcinoma of the breast. Immunohistochemical staining with anti-COX-2 antibody was performed in normal breast tissue (n = 15), usual ductal hyperplasia (n = 15), ductal carcinoma in situ (n = 30), and invasive ductal carcinoma (n = 99). Expression of COX-2 in invasive ductal carcinoma was correlated with immuno histochemical expression of HER-2/neu and p53 protein as well as clinicopathologic features.

COX-2 expression was found to be progressively elevated along the continuum from normal tissue to invasive ductal carcinoma ($p < 0.001$). COX-2 expression in invasive ductal carcinoma significantly correlated with tumor size ($p < 0.05$) and TNM stage ($p < 0.05$). COX-2 expression also significantly correlated with p53 and HER-2/neu protein expression (p,0.05 and p,0.001).

On multivariate analysis, only TNM stage and elevated COX-2 expression correlated with survival. Our results suggest the COX-2 may be involved in the carcinogenesis of the breast and may be an independent prognostic indicator in patients with invasive ductal carcinoma. HER-2/neu and p53 are likely to be involved in the regulation of COX-2 expression in invasive ductal carcinomas of the breast.

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Control of estrogen receptor by tumor suppressor protein PTEN in breast cancer cells

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Background: Estrogen receptors (ERs) mediate the biological effects of estrogen in mammary cells by binding to estrogen response elements in the promoter region of target genes or through protein-protein interactions. Anti-estrogens such as tamoxifen inhibit the growth of ER- positive breast cancers by reducing the expression of estrogen-regulated genes. Recent studies show that PI3-Kinase/Akt is involved in anti-estrogen –resistance in ER-positive breast cancers. However, tamoxifen-resistant growth of ER-positive tumors remains a significant clinical problem. Here we show that novel tumor suppressor PTEN, anti-PI3-Kinase stimulates expression of ER in ER-positive breast cancer cells, and PTEN is up-regulated by estrogen.

Methods: MCF-7 cells were treated with 17β-estradiol, and PTEN expression was determined by western-blot analysis. To investigate PTEN effects on ERs in breast cancer cells, MCF-7 cells were infected with vector alone (Ad/LacZ) or PTEN viral vector (Ad/*PTEN*) for 72 hours.

Results: Estadiol strongly induced PTEN expression in MCF-7 cells. Treatment of MCF-7 cells with Ad/*PTEN* increased significant PTEN level and induced an increase in ER expression.

Conclusion: These results suggest that ER is an important mediator of expression of the tumor suppressor protein PTEN in breast cancer cells, and in contrast ER is a target of PTEN. These studies form the basis for further investigations to improve the anti-tumor effects of tamoxifen against breast cancers.

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Hyperphosphorylation of translational repressor 4E-BP1 as prognostic factor in human breast cancer

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Background: Activation of the PI3K/Akt/mTOR signal transduction pathway is mainly dependent of membrane receptors and contributes to the development and progression of tumors by prevention of apoptosis and deregulation of cell cycle in a broad spectrum of human tumors. mTOR controls the mammalian translation machinery and constitutes a main controller in cell growth.

Methods: We have analyzed 103 human primary breast tumors with a complete immunohistochemistry (IHC) profile including multiple membrane receptors and phosphorylated (p) signaling proteins: HER2/neu, EGFR, p42/44MAPK, Akt, 4E-BP1, eIF4G, p70S6K, S6 and Ki67. Proposed biomarkers were validated in a subset of both frozen and paraffin-embedded breast tumors by Western blot and IHC.

Results: Activation of PI3K/Akt/mTOR signaling cascade was significantly detected in a high proportion of breast tumors (41.9%). Patients with HER2/neu overexpression showed a higher activation of Akt compared to negative ($p < 0.001$) and levels of pAkt were correlated with its downstream molecules p4E-BP1 ($p = 0.001$) and pp70S6K ($p = 0.05$). Interestingly, p4E-BP1 was mainly expressed in poor differentiated tumors ($p < 0.001$), significantly correlated with tumor size ($p < 0.001$) and with presence of lymph node metastasis ($p = 0.002$). Finally, majority of tumors with p4E-BP1 showed an increased rate of loco-regional recurrence ($p = 0.002$).

Conclusions: In breast cancer, activation of major cellular signaling pathways is partially mediated by overexpression of membrane erbB receptors, but frequently, activation of signaling proteins is not just dependent of these receptor signals. Evaluation of activation of the converging downstream signaling proteins, as 4E-BP1, could be a stronger prognostic indicator regardless upstream oncogenic alterations. In this study, we show that hyperphosphorylation of 4E-BP1 in breast cancer is associated with high grade, tumor size, lymph node metastasis and loco-regional recurrences.

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Clinical usefulness of ATBF1-A expression in breast cancer as a prognostic and predictive marker

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Purpose: The AT motif-binding factor 1 (ATBF1) gene was first identified as a suppressor of the alpha-fetoprotein (AFP) gene through its binding to an AT-rich enhancer element of this gene. The gene is located at chromosome 16q22.3-q23.1 where loss of heterozygosity has been observed in various malignant tumors, especially in breast cancer. This led us to hypothesize that there was a link between levels of ATBF1 expression and the metastatic potential of breast cancer and also, therefore, the prognosis of these patients.

Experimental design: In the present study, the level of ATBF1-A mRNA expression was analyzed using quantitative real-time reverse transcriptase-PCR, in 153 female patients with invasive carcinoma of the breast. ATBF1-A protein expression was also determined by immunohistochemistry from available 90 cases of paired tissues. An association was sought between ATBF1-A expression and various clinicopathologic factors.

Results: ATBF1-A mRNA was expressed at significantly higher levels in breast cancer patients with no axillary lymph node involvement, with small tumors and in estrogen receptor positive tumors. By contrast, no relationship was found between ATBF1-A protein expression and any of the other clinicopathologic factors. Patients expressing high levels of ATBF1-A mRNA tended to have a better prognosis than those expressing low levels. Univariate and multivariate prognostic analyses showed that ATBF1-A mRNA expression is an independent prognostic factor for disease-free survival. Additionally, cytoplasmic expression of ATBF1-A protein tended to be seen in the hormone responsive tumor.

Conclusions: In breast cancer, levels of ATBF1-A mRNA may serve as a predictive indicator of lymph node metastasis. ATBF1-A gene expression may have potential both as a marker of endocrine responsiveness and also as a prognostic indicator for breast cancer progression.

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Tamoxifen induced estrogen receptor activity in endocrine resistant breast cancer

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Introduction: ER co-activator, AIB-1, is associated with decreased disease free survival in breast cancer.

Aims: In breast cancer, though most ER positive patients will initially respond to endocrine treatment, many will eventually relapse. Inappropriate