Persistent activation of the transcription factor STAT3 has been detected in many types of cancer including breast cancer. Activated STAT3 plays an important role in tumor progression and metastasis. To analyze the molecular mechanisms of persistent STAT3 activation we coexpressed single-labeled STAT3-CFP or double-labeled STAT3-CFP-YFP (STAT3-CY) with v-Src resulting in constitutive tyrosine phosphorylation and nuclear accumulation of the fluorescent transcription factors. We found that activation of STAT3 by v-Src requires the intact SH2-domain of STAT3 but is insensitive to deletion or inhibition of Janus kinases (Jaks) and overexpression of SOCS3. By bleaching selectively the YFP moiety of STAT3-CY in the cytoplasm and by monitoring the distribution of the CFP and YFP fluorescence over time, we show that persistently activated STAT3 shuttles constitutively between cytoplasm and nucleus. Computational evaluation of the data by model-based parameter estimations revealed that activated STAT3 shuttles more rapidly than non-activated STAT3. Inhibition of exportin-1-mediated nuclear export results in reduced STAT3 shuttling more rapidly than non-activated STAT3. Inhibition of exportin-1-mediated nuclear export results in reduced tyrosine phosphorylation of STAT3 and decreased induction of target genes such as cyclin D1. We identified inhibition of nucleocytoplasmic shuttling of persistently activated STAT3 as a new target for the treatment of cancer.

3

Real time analysis of HER2–PI3K/AKT pathway in breast carcinoma samples
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Despite the recent progress in early detection, surgery and treatment, breast cancer continues to be a major cause of female mortality in western countries. A major breakthrough in tumor patient treatment has been achieved in recent years by the advent of target driven drugs such as Trastuzumab, a humanized recombinant antibody to HER2/neu receptor. Nevertheless it is now clear that cell proliferation and cell survival in breast cancer is a complex process involving steroid hormones, growth factors and their receptors. The understanding of the signaling pathways involved in breast cancer tumorigenesis may help to find new predictive factors and develop new strategies for treatment. Recent evidences have suggested that the PI3K/Akt pathway may play a major role in breast cancer tumorigenesis and at the same time could represent an ideal target for novel drugs. In fact, the PI3K/AKT cascade is a key signaling pathway involved in cell proliferation, survival and growth. Alterations in the PI3K/Akt signaling cascade are common in human cancers and result in hyperactivation of the pathway leading to tumor progression. Somatic mutation of the PIK3CA gene, encoding the p110 alpha catalytic subunit, may occur in a variety of cancers; in breast carcinoma the reported mutation frequency ranges from 18% to 40% of cases. These mutations have been reported as activating mutations causing an increased lipid kinase activity. The biologic and clinical relevance of this genetic event is still poorly understood. Recent evidences produced in our lab, suggest that the PIK3CA mutational status does not influence the overall pattern of expression of the downstream genes involved in the PI3K/AKT pathway such as PTEN, AKT2 and mTOR. It also appears that the PIK3CA mutations do not interfere with the transcriptional activity of the gene itself. The expression levels of HER2/neu, AKT2, and mTOR are significantly correlated only in the HER2/neu negative cases (IHC score: 0–1). The high mRNA levels of HER2/neu do not influenced the mRNA expression pattern of the downstream genes in the cascade. The activity of AKT2 and mTOR in HER2 overexpressing cases appears then to be regulated mainly at post-translational level.

4

Estrogen receptors and breast cancer cells migration
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Objective. – Estradiol enhances breast cancer cell division and cancer growth, but few data are available on the effects of this steroid on breast cancer cell migration, invasion and metastasis.

Design. – We used estrogen receptor (ER)-positive or ER-negative human breast cancer cells to study the effects of estrogens or anti-estrogens on cell migration and invasion and to check the molecular events that mediate these actions.

Results. – In T47-D ER+ cells, estradiol induces a rapid remodeling of cortical actin associated with horizontal migration and invasion of three-dimensional matrices. These actions are not found in ER-cells (MDA-MB-468). These effects are mediated by a time-consistent activation of the actin-binding protein, moesin that is dynamically phosphorylated in ER+ breast cancer cells. Moesin activation by estradiol relies on the recruitment of a cell-membrane estrogen receptor-α (ERα), while ERβ is not involved in this process. Activated ERα interacts with Gα13 at the cell membrane, and activates the signaling to RhoA and Rho-associated Kinase 2 (ROCK-2), that phosphorylates moesin. Knock out of ERα, Gα13 or RhoA with different techniques results in prevention of moesin activation and actin remodeling. Co-treatment with selective ER modulators such as ICI 182,780 or tamoxifen blocks the effects of estradiol on all of these targets. Immunohistochemical ana-