Karolinska Institutet

**Department of Oncology-Pathology**

# Proteomics of Invasiveness of Human Breast Epithelial Cells

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i lecture hall, CCK R8:00

**Fredagen den 24 Februari, 2012, kl 10.00**

av
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# ABSTRACT

Mechanisms of malignant transformation and cancer invasion and factors controlling them in response to various stimuli remain elusive. We used proteomics and systems biology to explore these mechanisms in human breast epithelial cells. The objective has been to identify a set of biomarkers for diagnostics and prognostics of breast cancer.

Acquiring of high proliferation by cells is a major hallmark of malignant transformation. Using global expression proteome profiling approach, we identified a set of proteins associated with high proliferation rate of human breast epithelial cells upon carcinogenic transformation (paper I). In this study, we described a proteome signature of cells with enhanced proliferation rate, and observed that deregulation of CDK4 and cyclin D3 may be among the early malignant transformation events.

Distal metastasis is the leading cause of death among breast cancer patients, and invasion of cancer cells is the first step in metastatic process. We established a highly invasive clone of MCF7 cells from non-invasive MCF7 cells (paper II). Using proteome profiling, we identified key regulators of invasiveness. Systemic analysis suggested that the invasive-specific network has features of a scale-free network, with TGFβ, EGFRB, TAF1, HNF4α, MYC and RB1 as key nodes. Analysis of TGFβ and EGF-centered network showed more than 30 key nodes which may define how TGFβ and EGF cooperate. Among these nodes were identified insulin, VEGF, HNF4α and NFκB. This result indicates that the insulin signaling disturbance may interfere with the invasiveness, thus explain the clinical observation of the increased risk of breast cancer metastasis in diabetes patients.

The correlation between protein translation and breast cancer is crucial in understanding of breast carcinogenesis. In paper III, we identified eukaryortic elongation factor 1 A1 (eEF1A1) as a direct substrate of type I transforming growth factor-β-receptor (TβRI). We showed that the phosphorylation of eEF1A1 at Ser300 by TβR-I mediates a direct inhibitory effect of TGFβ on protein synthesis, and contributes to effects on cell proliferation, anchorage-dependent and anchorage-independent cell growth. Furthermore, we showed that the phosphorylation of Ser300 is decreased in human breast tumors. In paper IV, we showed that eEF1A1 itself contributed to the increased proliferation of human breast epithelial cells by promoting transition of cells through the S- and G2/M-phases of the cell cycle. Therefore, our identification of eEF1A1 as a substrate of TβR-I unveiled novel translation-related regulatory pathway downstream of TβR-I, which is involved in breast tumorigenesis.

Breast cancer metastatic suppressor I (BRMS1) was identified by us as an invasiveness-related protein. In paper V, we showed that expression of BRMS1 resulted in a shift to epithelial morphology of otherwise mesenchymal morphology MDA-MB-231 cells. Our study concluded that TGFβ and EGF may modulate BRMS1-dependent breast cancer invasion by regulating focal adhesion and cytoskeletal rearrangement, and that Smad2 and Erk1/2 phosphorylation are involved in molecular mechanisms engaged by BRMS1.

Thus, presented here studies delivered a proteome signature of invasiveness and enhanced proliferation, and explored roles of eEF1A1 and BRMS1 in breast tumorigenesis. We described proteome signatures and proteins which may be considered as markers for diagnostics and prognostics of human breast cancer.

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