

TYROSINE KINASE SIGNALLING NETWORKS IN HUMAN BREAST CANCER

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Gene expression profiling has led to a new molecular taxonomy of breast cancer, revealing at least 5 cancer subtypes: luminal A and B (both ER-positive), normal-like (now thought to be due to contamination by normal epithelium), erbB2 and basal (ER-negative). These subsets differ in their prognosis and their response to specific treatments, with the erbB2 and basal subsets exhibiting the shortest overall and relapse-free survival, the luminal A subtype displaying increased sensitivity to endocrine therapy and a proportion of erbB2 cancers demonstrating a selective response to trastuzumab. Further investigation of the molecular mechanisms underpinning development of these subtypes, together with their *de novo* or acquired resistance to therapy, will help refine existing phenotypes and identify both novel phenotypes and potential targets for the development of novel therapeutic strategies. To this end, we are utilizing both candidate-based and 'global' screening strategies to identify components of tyrosine kinase signalling networks that contribute to phenotype specification in breast cancer and act as markers of prognosis and therapeutic responsiveness.

Our recent research on basal breast cancers provides an example of this bilateral approach. Since these cancers lack ER, PR and erbB2 they are resistant to endocrine and trastuzumab therapy, and hence present a major treatment dilemma. Recent research has indicated that basal breast cancer cell lines exhibit enhanced sensitivity to the Abl/Src family kinase (SFK) inhibitor dasatinib. Interrogation of published transcript profiling databases, in combination with Western blot analysis of a wide panel of breast cancer cell lines, reveals that the SFK Yes exhibits similar expression in basal and luminal-type breast cancer cell lines, while Src and Abl are expressed at higher levels in the latter type. However, the SFK Lyn is predominantly expressed in basal-type cell lines. Consequently Lyn may play a role in specification of the basal lineage and the development of basal breast cancers. In parallel, we are undertaking phosphoproteomic profiling of human breast cancer cell lines by immunoaffinity purification of tyrosine-phosphorylated proteins followed by LC-MS/MS. Highlighting the power of this approach, analysis of MCF-10A basal mammary epithelial cells expressing activated (Y527F) Src has detected tyrosine phosphorylation of 125 proteins on a total of 223 phosphorylation sites. Identified tyrosine-phosphorylation sites include those on 'classical' Src substrates such as FAK, Cas, PAG/Cbp and cortactin, numerous sites listed in the Phosphosite database but not functionally characterized in this context (eg on EphB4, different plakophilin isoforms, delta-catenin) and novel sites (cadherin 3). Application of this approach to our cell line panel will complement the candidate approach and identify kinases with unsuspected roles in basal breast cancer. In addition, it will provide a novel insight into the molecular heterogeneity of basal breast cancers and identify potential markers for phenotypic refinement.