Understanding the Apoptotic Signaling Pathways In Breast Cancer Using Microarrays, Proteomics and Bioinformatics

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Abstract

Breast cancer is one of the most common causes of cancer death to women worldwide. In this study A431 cells, derived from epidermoid carcinoma, are used as a model to study breast cancer. This cell line over-expresses the Epidermal Growth Factor Receptor (EGFR/HER1) and when treated with a high dose of EGF will undergo apoptosis via the activation of EGFR/HER1 signaling. However, little work has been conducted to identify the underlying molecular mechanisms. The limited available data implicates components of the interferon response pathway as mediators of the apoptotic signal in cancer cells. The genetic network through which EGFR/HER1 can induce apoptosis is not known at the present time. With understanding of the genetic regulatory hierarchy and the molecular mechanisms linking EGFR/HER1 signaling and apoptosis, better drug targets that might regulate apoptosis in cancers that over express HERs can be identified. This thesis focuses on the hypothesis that an apoptosis specific signaling cascade can be triggered by HERs in A431cells. Activation of HER receptors has led us to identify downstream components by global analyses of gene expression and associated regulatory miRNAs and protein levels using microarray and proteomics platforms. A high dose of EGF leads to the induction of apoptosis in A431 cells by activating a number of pathways, which are known to promote apoptosis. These include the STAT pathway and downstream components including cytokines and suppressors of cytokine signalling, cleavage of serpinb1 into L-DNAaseII and down regulation of mutant TP53, which may perturb the cytoskeleton and cell adhesion proteins. Furthermore, our data showed that gene expression and proteomic data were quite different, with very little overlap in terms of transcripts and proteins. Therefore, we considered that post-transcriptional regulation might be crucial. MicroRNAs are known to be important post-transcriptional regulators; as a result, we sought to identify potential regulatory miRNAs with respect to induction of cell death by EGF. We identified novel interaction regulatory networks based on the crosstalk between miRNAs and mRNA/protein that resulted in the induction of apoptosis in A431 cells. We also identified a number of miRNAs that may play an important role in the regulation of apoptosis in A431 cells after EGF treatment. We have used bioinformatics databases and *in silico* analysis tools to create a comprehensive catalogue of genes/proteins that may induce apoptosis in A431 cell after EGF treatment.

We have shown that using various complimentary platforms including gene expression, miRNA expression, proteomics, and network prediction to analyse the induction of apoptosis in treated A431 cells, we have achieved a greater understanding of the molecular mechanisms at work. This in turn may provide strategies for combined therapies and/or predict novel potential mechanisms/targets for chemotherapy aimed at inducing cell death in tumor cells.

Declaration

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List of Publications contributed to during Ph.D candidature

Combined gene expression and proteomic analysis of EGF induced apoptosis in A431 cells suggests multiple pathways trigger

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