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Expression of HER2 in Breast Cancer Promotes a Massive Reorganization of Gene Activity and Suggests a Role for Epigenetic Regulation

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Amplification and expression of the HER2/ErbB2 oncogene in breast cancer occurs in 25-40% of cases and is associated with aggressive disease [1-4]. HER2/ErbB2 is a trans-membrane tyrosine kinase of the EGF (Epidermal Growth Factor) receptor family. The receptor readily dimerizes leading to autophosphorylation and activation of heterodimer partners [5-6]. These partners include EGFR/ErbB1/HER1, HER4 and especially HER3 [5-7]. The activated receptors in turn recruit adaptor proteins which sequester substrates for downstream activation. HER2 signals through at least four major pathways including the Map kinase, PI3K/Akt, Phospholipase C, and STAT pathways [8-11]. The Map kinase pathway leads to the activation of genes that promote cell proliferation. PI3K/Akt promotes down regulation of several intermediates of apoptosis thereby promoting increased cell survival. Together these effects provide a potential mechanism for the oncogenic role of HER2.

An important landmark in breast cancer therapy was the development of the humanized monoclonal antibody, Herceptin/Trastuzumab, directed against the extracellular portion of HER2. However, in spite of advances in targeted therapy and good responses with combined anti-HER2+/chemotherapy approaches, diverse mechanisms of resistance to treatment are apparent in breast cancers with amplified HER2 [12] and recurrence is common. A durable therapy has been elusive.

Detailed expression analysis studies have provided lists of potential genes whose transcript levels are influenced by HER2 [13]. This information is vital for understanding resistance, for devising new treatments, and for understanding how the aggressive properties of HER2+ breast cancer are achieved. We studied MCF7 breast cancer cells that either expressed large amounts of active HER2 or did not express HER2, as well as breast cancer cell lines with naturally amplified HER2; BT474 and MDA453. We performed whole genome expression analysis using U133 plus 2 arrays with ~54,000 probe sets. We compared these data to the distribution of RNA Polymerase II (POL II) bound to promoters and the adjacent exons. This data was obtained using chromatin immunoprecipitation (ChIP) with antibodies to POL II [14] and Agilent promoter arrays that contained multiple probes for ~17,000 genes. Thus, we were able to determine whether changes in POL II binding to genes were associated with activation or suppression of transcription. Unexpectedly, we observed that *de novo* expression of HER2 in MCF7 cells causes POL II interaction ($p < 0.05$) with 606 genes. 20% of these new interactions exhibited significant fold changes up or down ($p < 0.05$ and > 1.4 fold change). 678 other genes lost POL II binding upon expression of HER2. These observations indicate that HER2 promotes a massive rearrangement in the genes activated or suppressed by HER2.

We quantified the amount and location of POL II binding in each promoter region. Some genes had no POL II binding and tended to be among the genes that were not transcribed. Some genes had strong POL II binding in the promoters, and these genes also tended to be among those that were not transcribed. Finally, there were genes that had weak or intermediate binding of POL II, including genes where weak POL II binding was dispersed over the promoter and downstream of the promoter. This latter class was more often associated with transcribed genes. We speculate that this last class with dispersed POL II binding may represent actively transcribing POL II whereas those genes with strong POL II binding in the promoter but low expression might be poised for transcription but not actively transcribing.

A group of 734 genes had detectable POL II binding in all three cell types that have high levels of HER2 (MCF7 expressing HER2, as well as in the naturally high expressing BT474 and MDA453 cell lines), but not in MCF7 controls that do not express HER2. 42 of these genes were transcriptionally up regulated and 50 down regulated. Presumably, the down-regulated class that showed de novo POL II binding is the class where POL II was dispersed on the actively transcribing gene in MCF7 and became more localized on the promoter when gene expression was reduced. The transcription of 68 genes was validated by qPCR for all three cell lines and gave a correlation coefficients of 0.74 – 0.90 ($p < 0.01$) with the array data.

Approximately 55 of the 734 genes are known to be involved in breast cancer including 12 of the 92 genes differentially regulated with HER2 expression. 38 genes of 734 have been implicated in HER2 function including 12 of the 92 genes [15-17]. The rest are candidates as novel HER2-regulated genes.

MetaCore Pathway analysis was used to look for networks among the 734 genes. Significantly enriched groups included the estrogen receptor, the progesterone receptor, and the androgen receptor–associated networks as previously reported in breast cancer [18-20]. Many genes were associated with stem cell and progenitor cell control as indicated by networks centered on NFKB, OCT3/4, and Nanog. These three overlapping networks account for 207 genes out of 734 genes (28%) and include 20 out of 93 differentially transcribed genes. Thus, the role of stem cells proliferation in HER2-regulated breast cancer is highly suggested. This is consistent with the observations HER2-dependent growth in cell culture and *in vivo* models [12, 21-23]. Our data revealed up regulation of DNMT3A and HDAC2 in HER2+ cells, which is of particular interest because of their potential global epigenetic effects in breast cancer [24, 25].

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The approach we have taken here allowed the identification of a large number of genes that are transcriptionally altered with changes in HER2 expression, and also genes that are changed in their potential for transcription via changes in POL II binding and positioning within the gene.

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