

Gene Expression Profiling in Signaling-Deficient B Cells

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ABSTRACT

Phosphoinositide 3-kinase (PI3K) activation regulates growth and survival in many signaling systems, and has been implicated in tumorigenesis. Bruton's tyrosine kinase (Btk) acts downstream PI3K in a pathway required for B cell receptor (BCR)-dependent proliferation. Little is known about the gene expression programs linking the PI3K/Btk signaling pathway to cell cycle entry. We used DNA microarrays to determine what fraction of genes this pathway influences, and to investigate whether PI3K and Btk mediate distinct gene regulation events. As complete loss-of-function mutations in PI3K and Btk alter B cell subpopulations and may cause compensatory changes in gene expression, we used B cells with partial loss-of-function in either PI3K or Btk. The expression profile of about 11000 genes of wildtype B cells and B cells with diminished PI3K or Btk function, unstimulated or stimulated with anti-IgM, was measured using Affymetrix chips. The gene expression values were calculated from the raw microarray data using D-CHIP software to exclude unreliable "outlier" probe pairs attributed to variability in the discriminatory ability of oligonucleotide probes, chip quality and the hybridization. The resulting expression data were then analyzed for changes under different experimental conditions using cyberT, a web-based program using a Bayesian framework for analysis of variance. The results showed that about 95% of the gene expression changes were not significantly affected by reduced PI3K or Btk. Less than 1% of the genes were regulated by PI3K and Btk, less than 3% by PI3K alone and less than 2% by Btk alone. Further clustering analysis confirmed that PI3K and Btk share target genes, and that PI3K influences additional genes independently of Btk. Together, these data are consistent with PI3K acting through Btk and other effectors to regulate

expression of a critical subset of BCR target genes that determine effective entry into the cell cycle. One PI3K/Btk target gene, cyclin D2, was previously shown to be required for BCR-driven proliferation. Future studies are aimed at evaluating other target genes for a role in cell cycle entry.

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