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Influence on the transcriptome of Tec family kinases with special emphasis on Btk

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Abstract

Over the last decade, scientists all over the world have profoundly used gene expression profiling based on microarray. Affymetrix is considered as the one of the pioneer platforms in the field of microarray technology. In this thesis, the Affymetrix Genechip® arrays were used to study the transcriptome of Tec family kinases with special emphasis on Bruton's tyrosine kinase (Btk) in avian B-lymphoma DT40 cell-line and fruit flies (*Drosophila melanogaster*). Btk is a protein tyrosine kinase belonging to the Tec family of kinases (TFKs). Btk is involved in signal transduction of the B cell receptor (BCR) pathway and plays an essential role in B lymphocyte development and function. X-linked agammaglobulinemia (XLA) is a primary immunodeficiency disease caused by mutations in the *BTK* gene. We studied Btk-deficient DT40 avian cell line reconstituted with the human *BTK* gene in order to investigate whether the loss-of-function can be rescued by the gene substitution at the transcriptomic level. Differences in the gene expression pattern showed statistically significant changes between parental DT40 and all the Btk KO cell populations, irrespective of whether they are reconstituted or not. Our result showed clonal selection of Btk knockout and gene reconstituted cells.

Btk is highly conserved during evolution appearing in sponges and in *Drosophila melanogaster*. In Drosophila, *Btk29A* (*Btk family kinase at 29A*) is the sole kinase that represents the Tec family, and it is most similar to Btk itself in terms of overall homology. In fact, the protein product from the type 2 splice variant of this gene exhibits the highest homology to Btk among the five mammalian TFKs. The type 1 splice variant has a shorter N-terminus that is unique to *Drosophila* Btk29A. *Btk29A* displays a dynamic pattern of expression through the embryonic to adult stages. $Btk29A^{ficP}$ is a unique allele in that it is devoid of the type 2 isoform while leaving type 1 isoform intact. The $Btk29A^{ficP}$ mutants survive to the adult stage, exhibiting a copulation defect and reduced lifespan after eclosion. We compared Btk mutant flies with their revertant strains using microarray gene expression profiling in adult brain and larvae CNS in order to investigate whether the loss-of-function phenotype can be rescued at the transcriptomic level. The whole transcriptomic profile for the different sample groups revealed Gene Ontology patterns for lifespan abnormalities in adult head neuronal tissue, but not in larval stage. We also carried out cross-species comparison in Btk-deficient flies and mice, which showed no significant overlap of the transcriptomic changes. Our results suggest that the evolutionary conservation is confined to components of the proximal signaling, whereas the corresponding, downstream transcriptional regulation has been differentially wired.

In paper III (manuscript), we extended a previous study addressing the influence of another TFK, namely Itk, by investigating the influence of co-culture the interaction of $CD4^+$ and $CD8^+$ T cells, when they are separated or not in the context of T cell activation using expression profiling data. T cell activation is one of the important steps in the immune response where hundreds of genes and proteins play crucial roles in a highly organized manner. Activation of those genes in the immune system is dependent on the T cell receptor signaling pathway and its regulation of transcription. Our result shows 6% of the transcripts are influenced by the contact between $CD4^+$ and $CD8^+$ T cells. This finding is of general importance, since whenever T cells are cultivated, whether they are separated into subsets or not, influences the analysis of their transcriptomes.

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