

Systems biology-defined NF-kappaB regulons, interacting signal pathways and networks are implicated in the malignant phenotype of head and neck cancer*

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Aberrant activation of the nuclear factor kappa B (NF- κ B) pathway has been implicated as one of the critical signals to modulate a diverse program of genes and promote tumorigenesis and progression of head and neck squamous cell carcinoma (HNSCC). We previously showed that putative NF- κ B binding motifs are prevalent in promoters of the up-regulated gene clusters in a subset of HNSCC associated with wild type p53 genotype. However, it is not well understood how NF- κ B globally regulates differentially expressed genes in coordination with other signal pathways and networks that contribute to the heterogeneity in gene expression and phenotypes observed. To approach this problem, we have applied a statistical model, COGRIM (Clustering Of Gene Regulons using Integrated Modeling), which is able to integrate available information from multiple sources for prediction of the transcriptional regulatory modules in a complex biological system. The regulons are defined as the organized modules or gene programs with conserved regulatory elements under the control of NF- κ B family members and other transcriptional factors. Using this modeling method, we defined NF- κ B regulons which consist of 748 NF- κ B target genes, representing 59% of the differentially expressed genes in HNSCC cell lines. Among the 748 genes, 75 (10%) are known NF- κ B targets confirmed by previous publications, and 183 (24.5%) were conserved between human and mouse.

The connections between NF- κ B regulons, signal pathways and networks were then explored through a structured network knowledge-based approach. We identified RELA and NF κ B1 dominant networks and pathways, related to proinflammatory responses and cytokines, signal receptors and intermediate kinases, cell cycle, adhesion, death and protein modification components. Furthermore, we performed experimental validation to confirm that NF- κ B was able to modulate expression of the predicted NF- κ B target genes which are associated with signaling pathways. We showed that the expression of 15 genes were significantly induced by Tumor Necrosis Factor-alpha (TNF- α), including 6 genes, *ALDH1A3*, *IL1R2*, *ITGA2*, *ITGA5*, *LAMA3*, and *LAMB3*, which previously have not been identified as NF- κ B target genes. After knocking down NF- κ B family member *RELA* or *NF κ B1* individually by siRNA, a significant modulation of the gene expression was found. Finally, to further examine the binding activity of RELA and NF κ B1 specifically for the gene promoters, we designed oligonucleotides using sequences from the promoters of NF- κ B target genes. RELA or NF κ B1 exhibited a moderate basal and significant TNF- α inducible binding activity on the predicted promoter sequences of *IL8*, *IGFBP3*, *CDKN1A*, *LAMB3* and *ITAG5* genes.

In conclusion, we successfully predicted NF-kappaB regulons through COGRIM modeling and connected them into organized NF- κ B regulatory networks and pathways. Such analysis revealed the concerted activation of NF- κ B target genes or gene products, many of them previously identified as unrelated molecules. The analysis of NF- κ B regulons established a complex interaction with novel and previously identified pathways and networks, which were composed of genes differentially expressed by HNSCC tumor cells. This study enables us to generate new hypotheses for the basic mechanistic investigation related to NF- κ B activity, as well as for further identification of biomarkers and therapeutic targets in HNSCC and other cancers.