Integrated experimental and systems biology approach to the identification of transcriptional regulatory network of p63 transcription factor

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Motivation

In spite of the large amount of data deriving from high-throughput genomic technologies, understanding how genes and proteins are connected and operate within networks is still a biological challenge. We developed an integrated experimental and systems biology approach to identify the biological pathways and the direct targets of p63 transcription factor, using primary keratinocytes as a cell culture model. p63, a member of the p53 family, is essential for the development of various ectodermal structures including skin, but its mechanism of action remains largely unknown.

Methods

A retrovirus expressing an inducible p63 gene was generated by fusing the coding portion of p63 with a tamoxifen-responsive estrogen receptor. Identification of immediate-early genes upon p63 activation was achieved by a time-series microarray analysis. Significant gene expression profiles were obtained for 800 genes. To infer the network surrounding p63 gene, we developed an algorithm called TSNI (Time Series Network Identification). TSNI modeled the network as a system of ordinary differential equations based on relating changes in gene transcript concentrations to each other and to the external stimuli. TSNI ranked the genes according to the probability of being the direct targets of p63.

Results

We selected top 100 genes as to be significant targets of DNP63a gene. These genes were found to belong to cell cycle control, cell adhesion and keratinocytes differentiation pathways, in agreement with what should be the targets of p63 according to literature. To verify the putative p63 target genes, we measured global changes in gene expression in p63 knockdown keratinocytes versus wild-type and we performed a ChIp on chip analysis on custom Agilent array targets to validate the TSNI predicted genes as functional targets. Our novel approach identified a number of genes that are significantly regulated by p63 at early time points and are involved in cell cycle control, cell adhesion and keratinocytes differentiation. We are currently deciphering the molecular function of these essential genes in stratified epithelia.

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