IDENTIFICATION OF NEW AP-2ALPHA REGULATED GENES: A BIOLOGICAL AND BIOINFORMATIC APPROACH

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Motivation

AP-2 transcription factors are a family of developmentally regulated DNA binding proteins. They are encoded by five different genes (alpha, beta, gamma, delta and epsilon) but they share a very common structure in particular in the DNA binding domains. They can act as homo- or heterodimers. They bind to GC-rich DNA sequences apparently without any specificity for the different isoforms. AP-2 play relevant roles in growth, differentiation, adhesion and migration by regulating specific genes.

Methods

In order to identify new AP-2alpha-regulated genes we downmodulated AP-2 alpha expression in epithelial tumor cells by RNAi and we investigated gene expression by microarray analysis (Whole Human Genome 44K, Agilent).

Results

We found 719 differentially expressed genes in AP-2alpha siRNA-expressing cells compared with control cells (FC> 1.5 pv< 0.01): 308 upregulated - 411 downregulated.

We validated 14 of these genes by Quantitative Real Time PCR. We then analyzed the regulatory regions of all modulated genes looking for AP-2 alpha binding sites. To this end we identified 15kb regions located upstream of each protein-coding gene in human and mouse and analyzed them with a WUBLAST local alignment procedure in order to define conserved non-coding blocks (CNBs) between human and mouse with a putative regulatory role. We then performed a statistical analysis of oligonucleotide frequencies in these regions aimed at the identification of candidate binding sites for regulatory elements. In particular, for every possible 5 to 9 nucleotide long DNA motif, we identified a set of human genes, which contained one or more overrepresented motifs in the conserved upstream regions. We then filtered these sets of genes looking for overrepresented differentially expressed genes independently of their Gene Ontology annotations. In this way we were able to define many putative binding sites for AP-2 and make a list of other transcription factors, which could cooperate with AP-2. Remarkably enough, genes up and down regulated in our microarray experiments, showed highly divergent vocabularies of transcription regulators. As a test of our results we were able to confirm the binding of AP-2alpha to the regulatory regions of genes such as ENDOTHELIAL AND SMOOTH MUSCLE CELL-DERIVED NEUROPILINLIKE (ESDN), FAST KINASE (FASTK) and EPIREGULIN (EREG) by Chromatin Immunoprecipitation (ChIP). We are currently performing microarray analysis of AP-2gamma siRNA-expressing cells to reveal the gene expression profile of this isoform. Our future goal will be to identify possible isoform specific AP-2 binding motifs.

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