REGULATORY NETWORKS OF JAK2/STAT5 PATHWAY ACTIVITY IN MYELOPROLIFERATIVE NEOPLASIA CELL LINES

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Background
The JAK/STAT signal transduction pathway is frequently altered in hematological cancers and, in chronic myeloproliferative neoplasms (CMN) in particular, the JAK2/STAT5 pathway is most frequently involved.

Aims
1) Identify and characterize new STAT5 target genes that might be playing a role in STAT5-mediated oncogenesis and could represent new therapeutic targets in these CMN.
2) Study the existence of hypothetical regulatory networks centered on microRNAs.

Methods
Bioinformatic screening
Using bioinformatics tools and expression microarrays, 64 genes were predicted as transcriptional targets of STAT5.

Cell lines
We measured the expression level of these 64 genes by RT-qPCR in two cell lines: -M-07e cell line, where the activity of the JAK2/STAT5 pathway can be induced by IL-3. To inhibit the pathway we used a specific STAT5 inhibitor. -HEL cell line, where the pathway is constitutively activated owing to the presence of the V617F mutation in JAK2. Here we used tyrphostin AG490 (JAK2 specific inhibitor) to inhibit the pathway.

Chromatin immunoprecipitation assay (ChIP)
To validate the interaction between STAT5 transcription factor and gene promoters, a chromatin immunoprecipitation assay (ChIP) was performed with a specific anti-STAT5 antibody, and gene promoters were detected in the immunoprecipitated fraction by PCR.

MicroRNAs
Taqman Low Density Arrays (TLDA) were used to measure the expression of 667 microRNAs after JAK2/STAT5 induction, after treatment with JAK2 and STAT5 inhibitors.

Results
We have identified ten genes with at least two-fold change in expression levels when the pathway is either induced or inhibited. We also identified 48 microRNAs that could regulate post-transcriptionally these ten genes and which show significant expression changes in these cell lines (Figure 1).

We have also confirmed that STAT5 binds to the promoter of six of these ten genes after IL-3 stimulation (Figure 2).

Figure 1: Network showing potential interactions between STAT5 target genes and microRNAs that could regulate them.

Figure 2: PCR analysis of interaction between STAT5 and its target gene promoters.

gDNA: genomic DNA.
Input: sample previous to immunoprecipitation.
IP C: sample immunoprecipitated with STAT5 corresponding to non-stimulated cells.
IP IL: sample immunoprecipitated with STAT5 corresponding to IL-3-stimulated cells.
C: negative control.

Conclusions
Our results provide new insights on the transcriptional program triggered by activation of the JAK2/STAT5 signaling pathway, which is constitutively activated in patients with myeloproliferative neoplasms. The identification of novel transcriptional targets of STAT5 and microRNA-target gene interactions will allow us to find novel regulatory loops which could lead to a deeper understanding of STAT5-mediated oncogenesis.

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