# A novel Rac1/PAK1/BCL6/STAT5 pathway modulates the expression of cell-cycle-associated genes in colorectal cancer cells



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#### Summary

Gene expression depends on binding of transcriptional regulators to gene promoters, a process controlled by signalling pathways. The transcriptional repressor BCL-6 downregulates genes involved in cell cycle progression and becomes inactivated following phosphorylation by the Rac1 GTPase activated protein kinase PAK1. Interestingly, the DNA motifs recognized by BCL-6 and STAT5 are similar. Because STAT5 stimulation in epithelial cells can also be triggered by Rac1 signalling, we asked whether both factors have opposing roles in transcriptional regulation and whether Rac1 signalling may coordinate a transcription factor switch. We used chromatin immunoprecipitation to show that active Rac1 promotes release of the repressor BCL-6 while increasing binding of STAT5A to a BCL-6-regulated reporter gene. We further show in colorectal cell lines that the endogenous activation status of the Rac1/PAK1 pathway correlated with the phosphorylation status of BCL-6 and STAT5A. Three cellular genes (cyclin D2, p15INK4B, SUMO1) were identified to be inversely regulated by BCL-6 and STAT5A and responded to Rac1 signalling with increased expression and corresponding changes in promoter occupancy. Together, our data show that Rac1 signalling controls a group of target genes that are repressed by BCL-6 and activated by STAT5A, providing novel insights into the modulation of gene transcription by GTPase signalling.

#### **Previous work: Rac1 Signaling Modulates BCL-6-Mediated Repression of Gene Transcription**



# New Data: The STAT5/BCL-6 transcriptional switch downstream of Rac1











(A) DLD-1 cells were co-transfected with the BCL-6 reporter and the indicated constructs. Note that STAT5 activates the BCL-6 luciferase reporter and, when combined with PAK1, reaches the stimulation levels normally induced by active Rac1, \*P<0.05 and #P>0.05. (B) Chromatin immunoprecipitation (ChIP) of the BCL-6 reporter promoter with anti ( $\alpha$ )-BCL-6,  $\alpha$ -STAT5 or a non-specific antibody (NS IgG) from DLD-1 cells transfected as indicated. Shown is a representative PCR of the precipitated promoter fragment quantities, together with the plot of quantitative PCR analysis of 3 independent experiments, \*P<0.05.

**Correlation of Rac1 signalling and activation** of PAK1, BCL-6 or STAT5 in CRC cell lines



STAT5 and BCL-6 regulation of the CCND2, CDKN2B and SUMO1 genes



Shown is the analysis of the SUMO1 gene in all three colorectal cell lines transfected as indicated (equivalent results were obtained for the other 2 genes – see bottom insert). Top panels show ChIP analysis of promoter occupancy by BCL-6 (black columns) or STAT5 (white columns) and middle panels the respective gene expression levels, \*P<0.05. Bottom panels show Western blot analysis of tranfected protein levels as well as the phosphorylation status of endogenous STAT5. Note that in SW480 depletion PAK1 siRNA or expression of KD PAK1 has no effect on promoter-bound BCL-6 whereas restoration of PAK1 expression leads to loss of BCL-6 from the promoter and an increase in gene expression. In the other two cell lines, inhibition of Rac1 or PAK1 are clearly correlated with more BCL-6 bound and less gene expression while activation of Rac1 or PAK1 promoted STAT5 binding and increased transcription.

**Current working model for the Rac1/PAK1/ BCL-6/STAT5** Pathway



(A) DLD-1 cells were transfected with Myc-Rac1-Q61L or control vector and analyzed by Western blot for the subcellular distribution of STAT5 between a soluble (S) and a chromatin-bound non-soluble (NS) fraction (detection of  $\alpha$ -tubulin and histone 2B served as controls). (B) Subcellular localisation of STAT5 determined by confocal fluorescence microscopy in DLD-1 cells co-transfected with DsRed-Rac1-Q61L and GFP-STAT5A. Shown is the overlay image of the DAPI (blue), GFP and DsRed channels. Note the nuclear STAT5 signal in Rac1-expressing red cells. In addition, DAPI and GFP signal intensities were measured along the indicated regions of interest (ROI, white lines). Note that nuclear GFP-STAT5 signal clearly increases when cells co-express active Rac1 (red cells, ROI 2 vs. green cells, ROI 1).

Equivalent lysate quantities of DLD-1, SW480 and HT29 colorectal cells were separated by gel electrophoresis and analysed by Western blot using the indicated antibodies to compare protein levels. The active Rac1 fraction was obtained by CRIB-pull down assays.

(A) CCND2, CDKN2B and SUMO1 gene promoter occupancy by BCL-6 and STAT5 was determined by ChIP in the three indicated cell lines. Shown are a representative PCR of the precipitated promoter fragments and a plot of the quantitative analysis of 3 independent experiments, \*P<0.05. A genomic fragment from the MUTYH gene was amplified to confirm the specificity of the precipitated target gene promoters. Note that BCL-6 binds predominantly in the PAK1-lacking SW480 cells and whereas a clear switch to STAT5 occurs in DLD-1 and HT29 cells with active Rac1/PAK1 signalling. (B) Expression analysis data for the selected genes in the 3 cell lines. Left panel shows representative RT-PCRs while quantitative PCR data is plotted in the right graph. RNA polymerase II (POL2) and phosphoglycerate kinase (PGK1) were amplified as control housekeeping genes.

# **Conclusion & Perspectives:**

- We identified a novel pathway through which Rac1 signalling can modulate the expression of a selected group of target genes.
- The next step will be the genome-wide identification of these genes to plot a profile of the impact of this pathway in the regulation of the cells transcriptome.

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