

ALTERNATIVE SPLICING OF TUMOUR-RELATED RAC1B IS REGULATED BY UPSTREAM SIGNALLING PATHWAYS



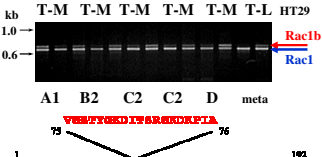
Vânia Gonçalves, Paulo Matos and Peter Jordan
Genetics Department, National Health Institute 'Dr. Ricardo Jorge', Lisbon, Portugal

SUMMARY The small GTPase Rac1 regulates signalling pathways controlling actin-dependent cell motility as well as gene transcription. An alternative splicing variant Rac1b is overexpressed in a subset of colorectal tumours and cooperates with mutant B-Raf to sustain tumour cell viability. The alternative splicing mechanism regulating Rac1b expression involves two antagonistic splicing factors, ASF/SF2 and SRp20. Using a Rac1 minigene approach and siRNA-mediated depletion, we identified ASF/SF2 as an enhancer of endogenous Rac1b splicing whereas SRp20 acts as a silencer. Inhibition of the PI3-kinase pathway increased protein levels of ASF/SF2 and promoted Rac1b generation. By contrast, depletion of endogenous protein kinase SRPK1 led to decreased Rac1b expression. Together, these data indicate that altered upstream signaling pathways in colorectal cancer cells will target splicing factors that regulate alternative splicing of the small GTPase Rac1.

Introduction to previous work

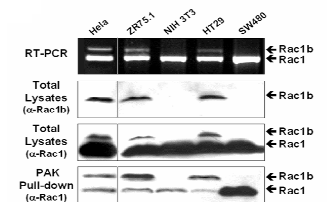
The Rac1b splice variant

Overexpressed in a subgroup of colorectal tumors (T) with B-RAF^{V600E}
[RT-PCR; (M:L = control tissue)]



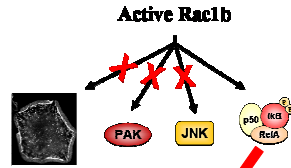
Jordan et al. (1999) Oncogene 18, 6835-6839

Endogenous Rac1b: little protein but highly active



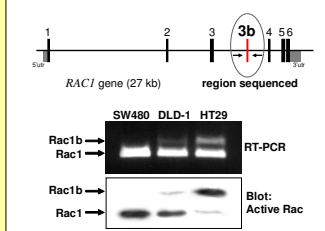
Properties of Rac1b:
• Increased GDP exchange rate *in vitro*
• Rho-GDI cannot downregulate Rac1b *in vivo*

Downstream signalling properties



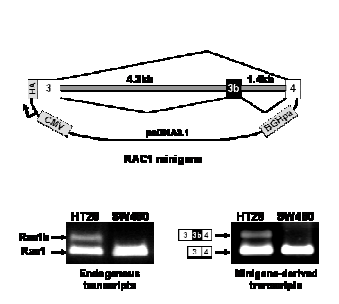
Promotes G1/S progression and cell survival
Matos & Jordan (2005) Exp Cell Res 305, 292-299;
Matos & Jordan (2008) Mol Cancer Res 6, 1178-84

Differences in Rac1b splicing between cell lines are not due to genomic mutations

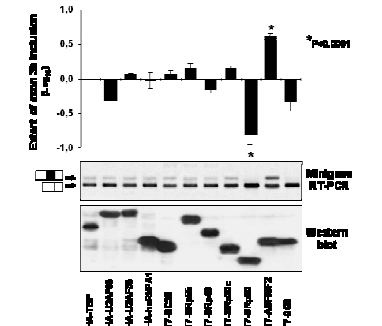


Which splicing factors are involved in the regulation of exon 3b inclusion/exclusion?

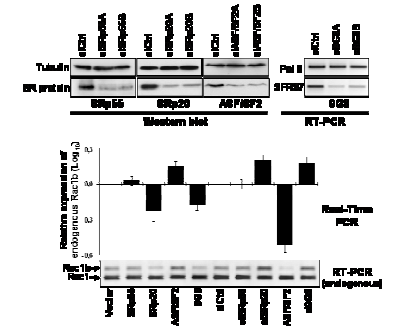
A RAC1 minigene reporter functionally reproduces the extent of alternative splicing observed in different colorectal cell lines



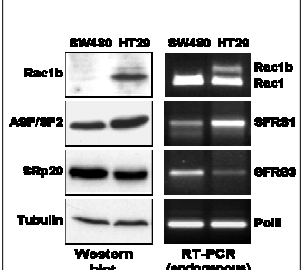
Overexpressed 9G8 and SRp20 decrease while ASF/SF2 increases exon 3b inclusion in HT29 cells



Depletion by siRNAs revealed SRp20 as a silencer and ASF/SF2 as an enhancer of alternative Rac1 splicing in HT29 cells

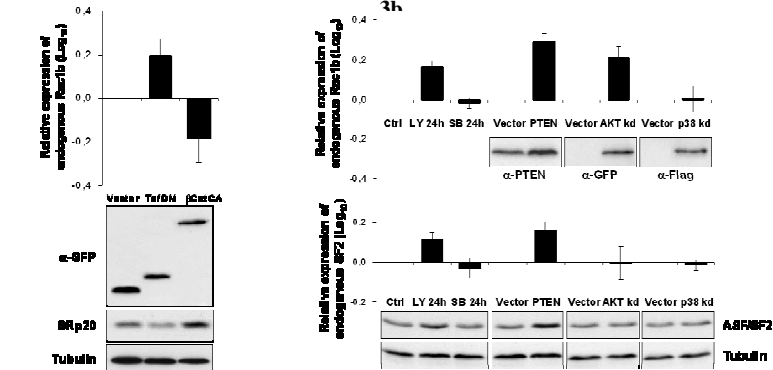


Endogenous levels of ASF/SF2 and SRp20 correlate with the amount of Rac1b expressed in different colorectal cell lines

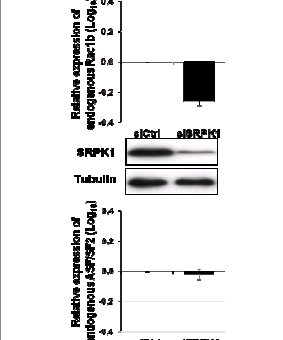


Which signalling pathways modulate the expression/activity of ASF/SF2 and SRp20?

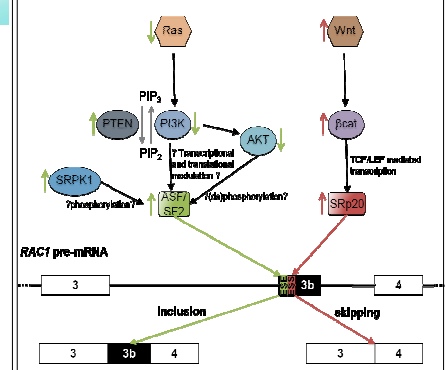
PI3 kinase and Wnt signalling pathways synchronize the expression of ASF/SF2 and SRp20, respectively, regulating inclusion or skipping of the alternative exon



Depletion of endogenous protein kinase SRPK1 led to decreased Rac1b expression



Current working model



Conclusions:

- The splicing factor ASF/SF2 acts as an enhancer of alternative Rac1 splicing whereas SRp20 acts as a silencer.
- The levels of ASF/SF2 and SRp20 transcript and protein correlate with Rac1b expression in colorectal cells.
- PI3 kinase and Wnt signalling pathways synchronize the expression of ASF/SF2 and SRp20, respectively, and together regulate whether alternative exon 3b is included or skipped during splicing of the Rac1 pre-mRNA.