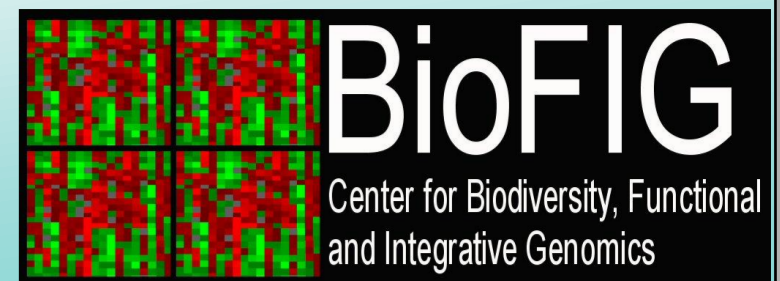


Phosphorylation of SRSF1 by SRPK1 regulates alternative splicing of tumor-related Rac1b in colorectal cells

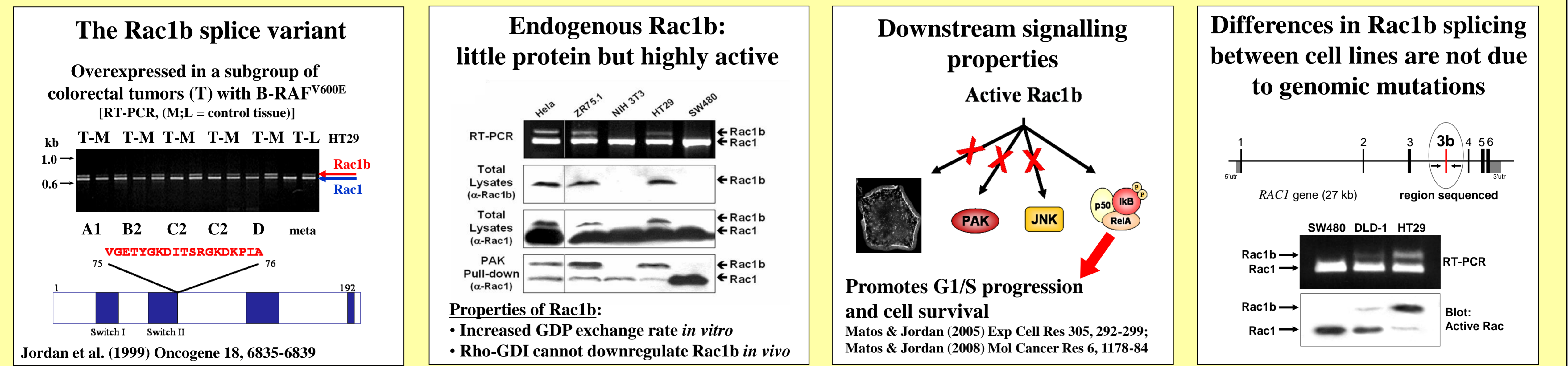


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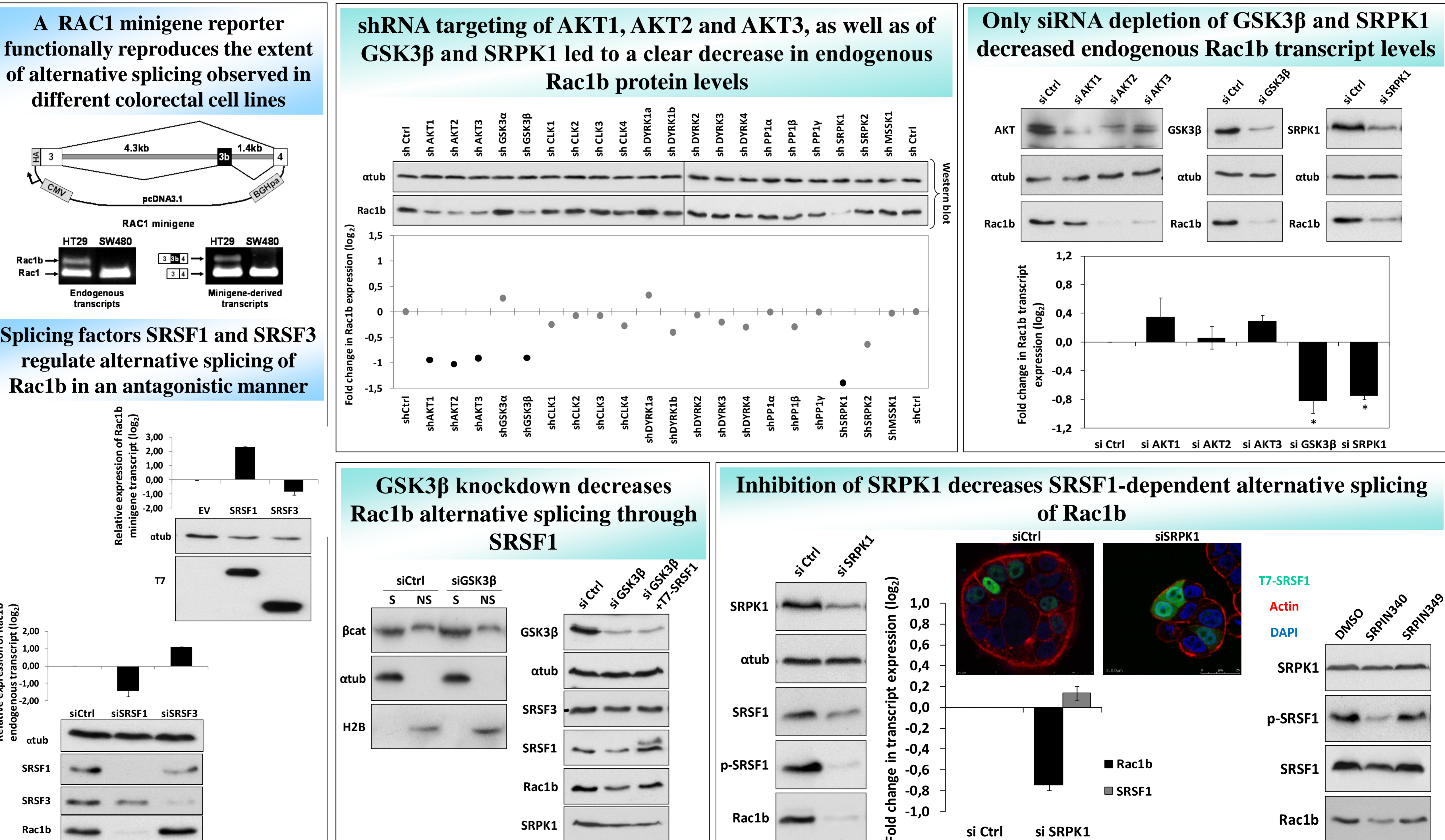


SUMMARY The pre-messenger RNA of the majority of human genes can generate various transcripts through alternative splicing, and different tissues or disease states show specific patterns of splicing variants. These patterns depend on the relative concentrations of the splicing factors present in the cell nucleus, either as a consequence of their expression levels or of post-translational modifications such as protein phosphorylation, which are determined by signal transduction pathways. Here we analyzed the contribution of protein kinases to the regulation of alternative splicing variant Rac1b that is overexpressed in certain tumor types. In colorectal cells we found that depletion of AKT2, AKT3, GSK3 β and SRPK1 significantly decreased endogenous Rac1b levels. Whereas knockdown of AKT2 and AKT3 affected only Rac1b protein levels suggesting a post-splicing effect, the depletion of GSK3 β or SRPK1 decreased Rac1b alternative splicing, an effect mediated through changes in splicing factor SRSF1. In particular, the knockdown of SRPK1 or inhibition of its catalytic activity reduced phosphorylation and subsequent translocation of SRSF1 to the nucleus, limiting its availability to promote the inclusion of alternative exon 3b into the Rac1 pre-mRNA. Altogether, the data identify SRSF1 as a prime regulator of Rac1b expression in colorectal cells and provide further mechanistic insights into how the regulation of alternative splicing events by protein kinases can contribute to sustain tumor cell survival.

Introduction to previous work



Which protein kinases regulate Rac1b alternative splicing, leading to its overexpression?



Conclusions:

- Depletion of AKT2, AKT3, GSK3 β and SRPK1 decreased considerably the levels of endogenous Rac1b.
- AKT2 and AKT3 knockdown appears to act only post-splicing, affecting solely steady state Rac1b protein levels in colorectal cells.
- GSK3 β and SRPK1 depletion decrease Rac1b alternative splicing by regulating this event through SRSF1.
- SRPK1 knockdown or activity inhibition leads to reduced SRSF1 phosphorylation and, consequently, reduced translocation to the nucleus limiting SRSF1 availability to enhance Rac1b alternative splicing.