

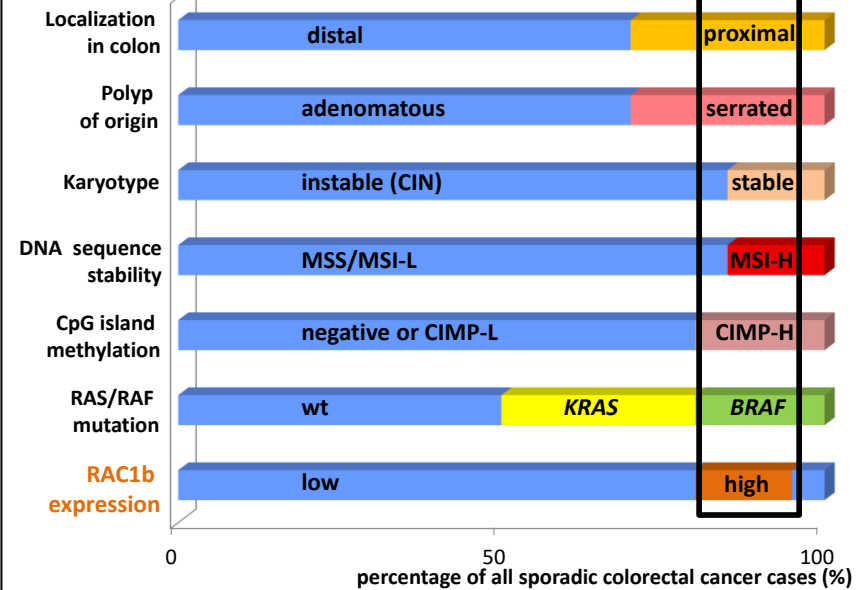


Tese de Mestrado
**Regulação do splicing alternativo
de RAC1b em células tumorais**

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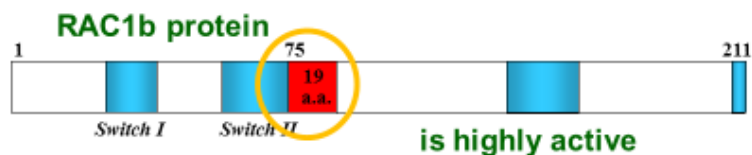
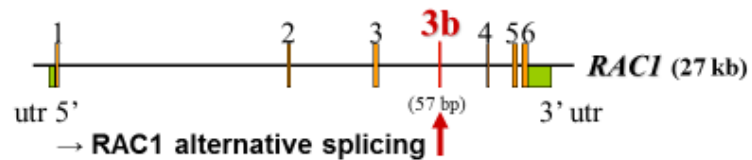
RAC1b overexpression characterizes a colon cancer subtype



Matos 2016, BBA-Rev Cancer 1866:51-63

RAC1b results from inclusion of exon 3b

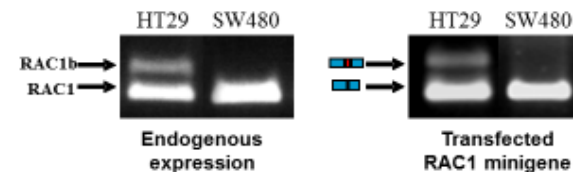
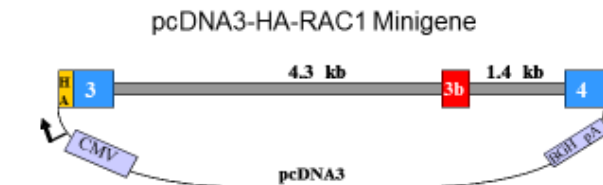
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How is RAC1b overexpression regulated at the splicing level?

4

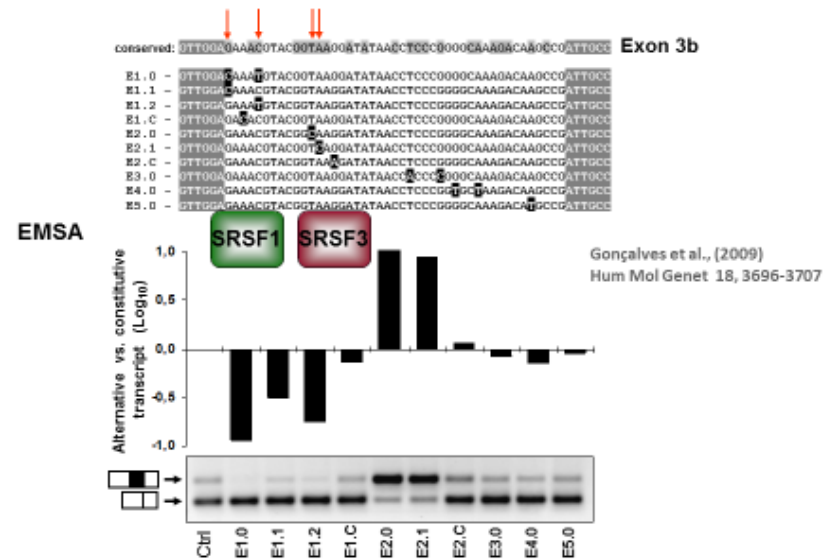
A minigene tool to dissect RAC1 alternative splicing



Used to identify regulatory sequence elements that determine exon 3b inclusion/exclusion

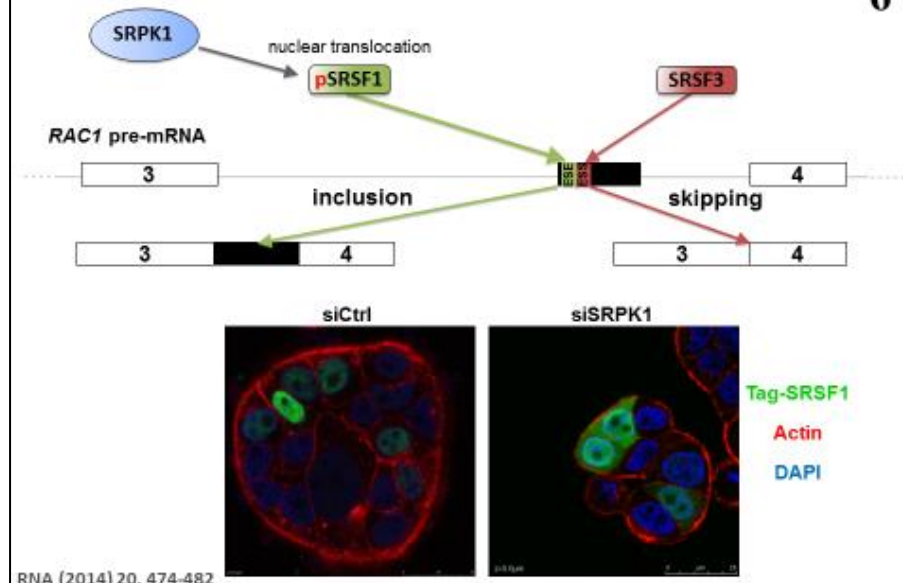
Two splicing factors identified that regulate exon 3b inclusion/exclusion

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Current model for regulation of RAC1 alternative splicing in colorectal cancer (CRC)

6

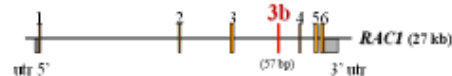


Take-home messages

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- Alternative splicing variant RAC1b is overexpressed in the BRAF mutant-subtype of colon cancer
- mRNA splicing factors determine the extent of alternative splicing and are regulated by signaling

...but evidence was reported that other splicing factors can participate



ESRP1 (Ishii JBC 2014, 289:27386-99) putative silencer in intron 3

PTBP1 (Hollander Genome Res 2016, 26:541-53) RAC1b enhancer, CRC

RANBP2 (Saitoh et al., 2012, MBC 23, 1115-28) affects nuclear SR protein import and splicing

What is their role and how do they work?

MSc project

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- Three potential splicing-regulatory factors will be tested
- What is their role and how do they work?

Work involves:

- DNA cloning and mutagenesis,
- culture of colorectal cells and their transfection with plasmids or siRNA,
- analysis by RT-PCR, Western blot and fluorescence microscopy