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Regulation of the alternative splicing of RAC1B in tumor cells

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Cancer is a molecularly heterogeneous disease and can present genetic modifications in different alternative pathways. The proposing laboratory has contributed to identify a subgroup of colon tumors, characterized by the simultaneous presence of an oncogenic mutation in BRAF and an overexpression of RAC1B, a splicing variant of GTPase RAC1 (1). Together, these two changes stimulate signaling pathways that promote the proliferation and survival of malignant cells (2).

Overexpression of RAC1B has also been identified in pancreatic, breast, lung and thyroid cancer. This protein could then be a therapeutic target for the treatment of patients with some types of cancer, which highlights the importance of studying its regulation in human cells.

But how does the overexpression of a splicing variant occur in tumor cells? The proposing laboratory found that there is an alternative exon 3b whose inclusion gives rise to the variant protein RAC1B with 19 additional amino acids and identified two splicing factors, SRSF1 and SRSF3, which promote and inhibit, respectively, the inclusion of this exon in colorectal cancer cells (3). More recently, the ESRP1 factor has also been studied in these cells, and has been found to promote RAC1B expression (unpublished data).

The present MSc's work will analyze whether the 3 factors mentioned above are also modulators of the alternative splicing of RAC1B in breast and lung cancer cells. The levels of expression of RAC1B and the 3 splicing factors will first be compared, in various breast and lung cell lines, using RT-PCR and Western blot techniques. As these factors are already cloned into expression vectors, they will be co-transfected individually into breast and lung cancer cells with a RAC1 minigene to determine their effect on exon 3b splicing. In parallel, the endogenous expression of these genes will be manipulated by RNAi to determine the effect on the endogenous transcript of RAC1B and the corresponding protein.

The results will contribute to a better understanding of the cellular specificity of alternative splicing mechanisms in general, and to the regulation of RAC1B in breast and lung cancer in particular.

Bibliography:

(1) Matos et al (2003). J. Biol. Chem. 278, 50442-50448.
(2) Matos et al (2008). Gastroenterology 135, 899-906.
(3) Gonçalves et al (2009). Hum. Mol. Genet. 18, 3696-3707.