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Área(s) temática(s): Assinalar a(s) que se aplica(m)

- Doenças crónicas degenerativas e genéticas
- Doenças infecciosas
- Epidemiologia, bioestatística e bioinformática
- Imunologia
- Promoção da saúde
- Saúde ambiental e da alimentação
- Serviços de saúde

Title

Lessons from Colon Cancer: How Signal Transduction Pathways Regulate Alternative Splicing of RAC1b

Authors, Affiliations

Gonçalves, V^{1,2}; Matos, P^{1,2,3}; Henriques, AF^{1,2}; Pereira, JF^{1,2} and Jordan, P^{1,2}

¹Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa

²BioISI - Biosystems & Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa

³Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa

Abstract

In colon cancer distinct genetic subtypes have been described, one of which involves overexpression of RAC1b, a variant generated by alternative splicing. Aberrant splicing is known to occur in cancer and can be caused by mutation in a gene or splicing factor but also represent a dynamic response to oncogene-induced cellular signaling and in this case it may be pharmacologically targeted. Here we explore how signaling pathways are involved in the deregulation of alternative RAC1b splicing in tumor cells. HT29 colorectal cells represent serrated colorectal tumors with *BRAF* gene mutation V600E in one allele and RAC1b overexpression. Cells were transfected with shRNA vectors directed against target candidate protein kinase transcripts and their effects on RAC1b levels analyzed 24h later by Western Blot and qRT-PCR. Treatment with kinase inhibitors or anti-inflammatory drugs was performed 24h prior to cell lysis. Because cell signaling involves protein kinases that can be targeted by inhibitory drugs, 20 candidate splicing-regulatory protein kinase genes were depleted by RNAi in HT29 cells. Two kinases, SRPK1 and GSK3 β , were found required to sustain RAC1b levels and both were shown to act upon the phosphorylation of splicing factor SRSF1, which binds to and promotes the inclusion of the alternative exon in RAC1b. Reduced



SRSF1 phosphorylation decreased its nuclear translocation and concomitantly RAC1b splicing. This regulatory pathway is controlled by GSK3 β and was found to be inhibited specifically by the anti-inflammatory drug ibuprofen.

Together, our results demonstrate that oncogenic signal transduction pathways deregulate alternative splicing and this may be drug revertable.

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