RBFOX REGULATION OF ALTERNATIVE SPLICING EVENTS IN THE DEPENDENCE OF PHOSPHORYLATION

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RBFOX is a RNA Binding Protein that regulates alternative splicing (AS) of hundreds of mRNA targets in a cell. Disruption of RBFOX binding capabilities results in misregulation of alternative splicing events related to cancer and neurological diseases. Additionally, recent studies have suggested that RBFOX can be regulated by phosphorylation events, which could retain the protein in the cytoplasm. The protein WNK3 belongs to a serine-threonine kinase family protein with a classical function in ionic balance and blood pressure. WNK3 kinase was related to the splicing mechanisms mediated by RBFOX. For this project we aim to understand the dependence of WNK phosphorylation for the neuronal mRNA targets of RBFOX. Initially we have used computational annotation to select a subset of endogenous alternative splicing events regulated by RBFOX. We are defining RT-PCR conditions to detect all these events in a systematic way, upon RBFOX overexpression and knockdown in HEK293 cells. We will then investigate if these events are dependent on the present and phosphorylation by WNK3. We are also using biochemistry to clarify the mechanisms and sites of RBFOX phosphorylation by WNK3. In addition, we aim to test small chemical molecules to inhibit the WNK3 kinase and to evaluate their affect on RBFOX targets. These small molecules are being produced as part of a collaboration with the Structural Genomics Consortium (SGC) and could have a potential biological use for research and treatment purposes.

Key words: alternative splicing;RBFOX, RNA regulation, Phosphorylation