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Analysis of Alternative Splicing Regulated by Ca^{2+} Signaling

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Summary

Alternative splicing achieves a numbers of protein expression more than those encoded in its genome. The number of alternative splicing increases as living organism becomes complexity. However, the mechanism of alternative splicing is still largely unknown. Therefore, uncovering the mechanism of alternative splicing may give a key role for the investigation of human genetic diseases and establishment of their therapy.

CHERP is first identified as Ca^{2+} signaling related protein in the endoplasmic reticulum. Then, is also found that CHERP is also localized in the nucleus, suggesting CHERP has another role in the nucleus. Here, I examined the CHERP interacting proteins by immune precipitation and the genes regulated by CHERP to elucidate whether CHERP has a role for the alternative splicing. CHERP interacting proteins were precipitated using Flag tagged CHERP and M2 antibody, and analyzed by mass-spec. In the absence of Ca^{2+}, SF3A and SF3B were most abundantly detected in addition to U2 SNP related proteins. By contrast, ribosomal and histone proteins were dominantly detected in the presence of Ca^{2+}. These results suggest that CHERP will have a role for the alternative splicing by the association with U2 snRNP and may alter the alternative splicing pattern through exchanging the associated proteins when Ca^{2+} signaling is activated. Next, the exon array analysis was performed to investigate the CHERP regulated exon. In the absence of CHERP by the siRNA mediated knock-down, the expression of more than thousands of exons were altered 2 fold and more. About a half of them were exon skipped and the rest of them were exon included. This observation suggests that CHERP affect the pattern of alternative splicing both positively and negatively. The mechanism of the alternative splicing regulated by CHERP is now on going.