

Alternative pre-mRNA processing and cancer therapeutics

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The inactivation or deregulation of apoptotic pathways can lead to tumor development and chemotherapy resistance. In recent years, the alternative pre-mRNA splicing of apoptotic factors has been given greater attention in cancer research as splice variants of a variety of specific signaling factors in apoptosis have an opposite/dominant-negative function, and dysregulation of alternative splicing is becoming a common characteristic of human cancer. In this seminar, the current status of alternative splicing and cancer therapeutics will be briefly overviewed. Following this overview, the seminar will focus on research from our laboratory demonstrating the cellular mechanisms by which the alternative splicing of caspase 9 is regulated in cells. Two splice variants are derived from the caspase-9 gene, pro-apoptotic caspase-9a and anti-apoptotic caspase-9b, by either the inclusion or exclusion of an exon 3, 4, 5, and 6 cassette. The alternative splicing of caspase 9 is highly dysregulated in non-small cell lung cancer (NSCLC) producing an anti-apoptotic phenotype. Studies by our laboratory identified a possible repressor RNA *cis*-element in exon 3 of the caspase 9 gene that regulates this mechanism. Using electromobility shift assays and mass spectrometry analysis, hnRNP A2/B1 (36kDa) and hnRNP L (65kDa) were identified as RNA *trans*-factors binding to this repressor *cis*-element. From these data, we hypothesized that hnRNP A2/B1 and hnRNP L are important regulators of caspase 9 pre-mRNA processing via repression of the inclusion of the exon 3, 4, 5, and 6 cassette. In this regard, we employed RNAi technology to examine the effect of downregulating hnRNP L and A2/B1 on the alternative splicing of caspase 9. Western blot analysis was first performed to determine the extent of hnRNP A2/B1 and hnRNP L downregulation by siRNA treatment. Indeed, siRNAs targeted against hnRNP A2/B1 and hnRNP L decreased hnRNP A2/B1 protein levels greater than 90%, and hnRNP L protein levels were decreased by 75%. Using these siRNAs, the effect of downregulating either hnRNP L or hnRNP A2/B1 on the alternative splicing of caspase 9 was examined. The downregulation of either hnRNP L or hnRNP A2/B1 resulted in a significant increase in the caspase 9a/9b ratio (enhanced inclusion of the exon 3, 4, 5, and 6 cassette). This study demonstrates that hnRNP L and hnRNP A2/B1 act as repressors of the inclusion of the exon 3, 4, 5, and 6 cassette of caspase 9 pre-mRNA. The significance of this research lies in numerous reports that both hnRNP L and hnRNP A2/B1 are early prognostic indicators of NSCLC, and are required for the survival of transformed cells. Furthermore, direct manipulation of the alternative splicing of caspase 9 affects the sensitivity of NSCLC cells to certain chemotherapeutic agents. Future studies will focus on determining the role of these hnRNPs in these biological processes in the context of the alternative splicing of caspase 9.