IDENTIFICATION OF ALTERNATIVELY SPLICED NOVEL TRANSCRIPTS OF HUMAN NEUROSERPIN IN BRAIN USING BIOINFORMATICS AND MOLECULAR BIOLOGY TECHNIQUES

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Pre-mRNA alternative splicing generates multiple mRNAs that code for different protein isoforms having similar, different or opposing functions. It is a highly regulated process that controls different pathways inside the cell. Identification of alternatively spliced isoforms is required in order to establish the functions of a gene. Neuroserpin is serine protease inhibitor that is mainly expressed in brain and is related to several diseases like dementia, epilepsy, Alzheimer's and FENIB. Neuroserpin inhibits various proteases like tPA, uPA, trypsin, NGF-γ, thrombin and plasmin. However, the inhibition efficiency of neuroserpin is different for these proteases. So far, only two alternatively spliced variants of neuroserpin have been reported. These variants differ only in their promoter region but code for the same protein.

In this study, we have identified alternatively spliced novel transcripts of human neuroserpin gene using a combination of bioinformatics and molecular biology techniques. The methodology involved prediction of novel exons with the help of gene and exon finding tools. To confirm the existence of these predicted isoforms, primers were designed from the novel region of exons and used in the subsequent RT-PCR experiments. The existence of the novel isoform was further confirmed by DNA sequencing. Western blot analysis was also performed to study the expression of isoforms at protein level. Since one of the novel transcript identified in this study was truncated at C-terminal, we performed site directed mutagenesis to introduce a stop codon in the neuroserpin gene cloned in pET28b(+) plasmid at desired position. Expression and protein purification was done in order to perform the structural characterisation of isoforms. Structural stability of novel isoform was also studied using MD simulation.