Transcriptional analysis of the human apoptosis-related BOK gene reveals novel, alternatively spliced messenger RNAs, a previously unknown 5´untranslated region (5´ UTR), and two new, shorter 3´ UTRs

P-01.2-03

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The BCL2 family includes pro- and anti-apoptotic members, basically differing in the combination of BCL2-homology (BH) domains. BOK is an apoptosis facilitator, yet an anti-apoptotic behavior has been described as well. This ambiguity could be attributed to the existence of multiple alternatively spliced transcripts encoding for distinct BOK protein isoforms. In this study, we describe the discovery of novel transcripts of the human *BOK* gene, most of which comprise new open reading frames (ORFs) and probably encode for new BOK protein isoforms. Moreover, we determined a new 5´untranslated region (5´ UTR) and two shorter 3´ UTRs. In brief, we started by performing bioinformatical analysis of publicly available expressed sequence tags (ESTs). Next, total RNA was isolated from 23 cancer cell lines originating from different human tissues and first-strand complementary DNA (cDNA) was synthesized starting from 5 μg of total RNA. PCR primers were designed to amplify only *BOK* cDNA-specific sequences and were used in two successive PCRs. Rapid amplification of cDNA ends (RACE) was used to study the 5´ and 3´ UTRs. Nested PCR and RACE products were electrophoresed on an agarose gel; bands of unexpected size were gel-extracted, purified, and sequenced using Sanger sequencing. We also performed next-generation sequencing (NGS) to unravel rare *BOK* transcripts. Our results led to the discovery of 21 novel *BOK* transcripts, 13 of which have distinct ORFs. *in silico* translational analysis revealed the putative existence of 7 novel BOK protein isoforms lacking internal peptides and possessing distinct C-termini. Moreover, we identified a previously unknown 5´ UTR, probably preceded by its own promoter, as well as two novel, shorter 3´ UTRs, with fewer post-transcriptional regulatory regions. Overall, the prospect of novel BOK protein isoforms and alternative UTRs raises questions about the role of this gene in both normal and pathological states and necessitates additional research.