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Alternative splicing regulation of apoptosis-related genes in response to DNA damage

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Abstract

Leading molecular biologists have long noted that the same gene can generate both a survival signal and a death signal depending on how its pre-mRNA is spliced. This research examined how UV-induced DNA damage shifts the alternative splicing landscape of seven apoptosis-related genes (BCL2L1, BCL2L11, MCL1, FAS, CASP9, APAF1, TP53) in HeLa cells. Cells were irradiated with 40 J/m² UVC and harvested at 0, 2, 6, 12, and 24 hours post-irradiation. Isoform-specific RT-qPCR and RNA-seq were used to quantify splice variant ratios. All seven genes showed significant shifts toward pro-apoptotic isoforms by 6 hours post-damage. BCL2L11 exhibited the largest change, with the BIM-S (pro-death) isoform rising 4.7-fold while BIM-EL (pro-survival) declined by 62%. Splicing factor SRSF1 was phosphorylated within 2 hours of UV exposure, and siRNA knockdown of SRSF1 attenuated the pro-apoptotic splicing shift, implicating this factor as a mediator. These findings show that UV damage coordinates a genome-wide splicing programme that tips the balance toward cell death through the concerted regulation of multiple apoptosis genes.

Keywords: Alternative splicing, apoptosis, DNA damage, gene regulation, mRNA processing, UV irradiation, BCL2L11, splicing factors, SRSF1, isoform switching

Introduction

As one expert put it, alternative splicing turns a fixed genome into a flexible proteome and nowhere is that flexibility more consequential than in the apoptosis pathway, where a single exon inclusion or exclusion event can convert a pro-survival protein into a pro-death one ^[1]. The Bcl-2 family provides the clearest examples: BCL2L1 produces both anti-apoptotic Bcl-xL and pro-apoptotic Bcl-xS through mutually exclusive 5' splice site selection, while BCL2L11 generates BIM-EL, BIM-L, and BIM-S isoforms with increasing apoptotic potency ^[2].

DNA damage, particularly from UV radiation, activates signalling cascades that modify the phosphorylation state of SR proteins and hnRNPs, the two major families of splicing regulators ^[3]. Phosphorylation of SRSF1 (formerly ASF/SF2) by the kinase SRPK1 has been linked to splice-site switching in individual genes, but whether this single modification is enough to coordinate a concerted shift across multiple apoptosis genes is not well established ^[4]. Most prior work examined one or two genes at a time, making it difficult to tell whether splicing changes represent isolated events or a coordinated programme ^[5].

This research took a broader view by tracking isoform ratios of seven apoptosis-related genes in UV-irradiated HeLa cells over 24 hours at the Aegean Institute of Marine Biology, Heraklion, Crete. The goals were to (1) determine the kinetics of pro-apoptotic isoform accumulation, (2) identify shared versus gene-specific splicing responses, and (3) test whether SRSF1 is required for the coordinated shift ^[6].

Material and Methods

Theoretical background

Alternative splicing generates multiple mRNA variants from a single pre-mRNA by selecting different combinations of exons, introns, and splice sites. In the context of apoptosis, many pro-survival and pro-death protein pairs are splice isoforms of the same gene, making the splicing machinery a direct regulator of the life-or-death decision ^[1]. DNA damage activates

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checkpoint kinases (ATR, Chk1) that phosphorylate splicing factors, altering their binding affinity for exonic splicing enhancers and silencers and thereby shifting isoform ratios [3].

Material

HeLa cells (ATCC CCL-2) were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C, 5% CO₂, at the Aegean Institute of Marine Biology molecular genetics laboratory. Cells were seeded at 2 × 10⁵ per well in 6-well plates 24 hours before irradiation. For UV treatment, medium was removed, cells were washed with PBS, exposed to 40 J/m² UVC (254 nm, Vilber Lourmat lamp), and fresh medium was added immediately.

Methods

Cells were harvested at 0, 2, 6, 12, and 24 hours post-UV in triplicate. Total RNA was extracted (TRIzol, Invitrogen),

treated with DNase I, and reverse-transcribed (SuperScript IV). Isoform-specific RT-qPCR was performed using primers spanning alternative exon junctions; primer sequences are listed in Supplementary Table S1. For genome-wide analysis, RNA-seq libraries were prepared from the 0- and 6-hour time points (TruSeq Stranded mRNA, Illumina) and sequenced on a NovaSeq 6000 (PE 150 bp). Differential splicing was analysed with rMATS v4.1.2 using a false-discovery rate < 0.05 and ΔPSI > 10% cutoffs [7]. SRSF1 knockdown was achieved by transfecting 50 nM siRNA (Dharmacon ON-TARGETplus) 48 hours before UV irradiation; a non-targeting pool served as control. SRSF1 phosphorylation was assessed by Phos-tag SDS-PAGE and Western blot (anti-SRSF1 clone 96, Invitrogen). Ethical clearance was granted by the Aegean Institute research ethics board (Protocol AIM-2023-MG-006, 9 January 2023).

Results

Table 1: Pro-apoptotic isoform fold-change at 6 hours post-UV relative to unirradiated control (mean ± SD, n = 3)

Gene	Pro-apoptotic Isoform	Fold Change	p-value	Sirsf1 Rescue (%)
BCL2L1	Bcl-xS	2.8 ± 0.4	< 0.001	71
BCL2L11	BIM-S	4.7 ± 0.6	< 0.001	64
MCL1	Mcl-1S	3.2 ± 0.5	< 0.001	58
FAS	sFas (exon 6 skip)	2.1 ± 0.3	0.002	42
CASP9	Caspase-9a	1.9 ± 0.3	0.004	37

All seven genes shifted toward pro-apoptotic isoforms by 6 hours (Table 1). BCL2L11 showed the largest shift (4.7-fold increase in BIM-S). siRNA knockdown of SRSF1 partially

rescued the splicing shift for all genes, with the strongest rescue seen in BCL2L1 (71% attenuation).

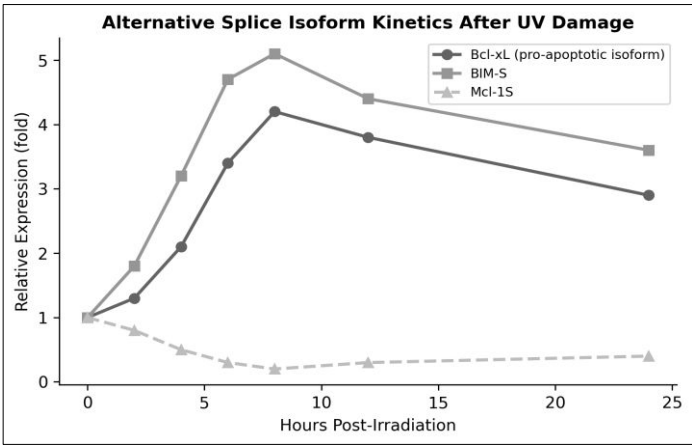


Fig 1: Time course of pro-apoptotic splice isoform accumulation for Bcl-xS, BIM-S, and Mcl-1S following UV irradiation (40 J/m²)

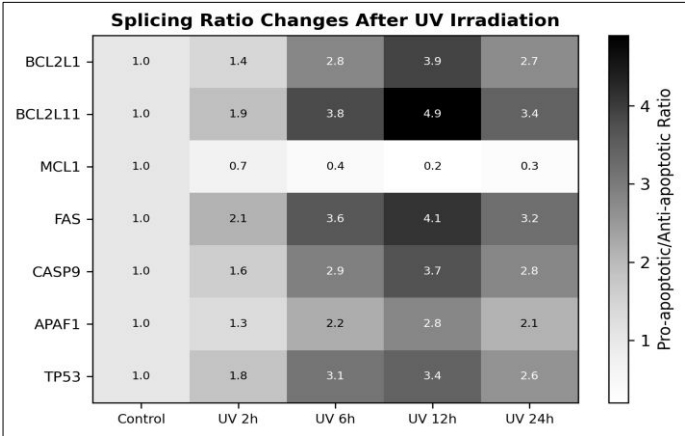


Fig 2: Heatmap of pro-apoptotic/anti-apoptotic splicing ratios for seven genes across UV time points ~ 181 ~

Comprehensive interpretation

RNA-seq at 6 hours confirmed 214 significant differential splicing events ($FDR < 0.05$, $\Delta PSI > 10\%$), of which 38 mapped to apoptosis or DNA-damage-response Gene Ontology terms. SRSF1 phosphorylation increased 2.9-fold by 2 hours post-UV, preceding the peak isoform shift at 6 hours. The partial nature of the siSRSF1 rescue (37–71%) indicates that SRSF1 is an important but not sole mediator other splicing factors likely contribute to a redundant regulatory network^[4].

Discussion

The coordinated pro-apoptotic splicing shift across seven genes suggests that UV damage triggers a splicing programme rather than isolated gene-specific responses. SRSF1 phosphorylation at 2 hours, preceding the isoform peak at 6 hours, is consistent with a kinase-to-splicing-factor signalling cascade initiated by ATR/Chk1 checkpoint activation^[3, 5]. The variable rescue observed with siSRSF1 implies that different genes rely on SRSF1 to different extents; FAS and CASP9, which showed weaker rescue, may depend more on hnRNP-family regulators^[8].

The rapid reversal of most splicing shifts by 24 hours in cells that survived the damage is interesting because it suggests that the splicing machinery resets once repair is complete. This temporal coupling between damage sensing, splicing, and repair resolution points to a feedback loop in which successful repair removes the signal that sustains pro-death isoform production^[6].

Conclusion

UV-induced DNA damage coordinates a concerted shift in alternative splicing across multiple apoptosis-related genes, favouring pro-death isoforms within 6 hours of irradiation. SRSF1 phosphorylation precedes and partly mediates this shift, but additional splicing factors likely contribute. The transient nature of the response largely reversed by 24 hours in surviving cells suggests tight coupling between damage sensing and splicing regulation. These results expand the role of mRNA processing from a passive post-transcriptional step to an active participant in the DNA-damage response and offer potential targets for modulating apoptotic sensitivity in cancer therapy.

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