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Mini-review

Splicing factors of SR and hnRNP families as regulators of apoptosis in cancer

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ABSTRACT

SR and hnRNP proteins were initially discovered as regulators of alternative splicing: the process of controlled removal of introns and selective joining of exons through which multiple transcripts and, subsequently, proteins can be expressed from a single gene. Alternative splicing affects genes involved in all crucial cellular processes, including apoptosis. During cancerogenesis impaired apoptotic control facilitates survival of cells bearing molecular aberrations, contributing to their unrestricted proliferation and chemoresistance. Apparently, SR and hnRNP proteins regulate all levels of expression of apoptotic genes, including transcription initiation and elongation, alternative splicing, mRNA stability, translation, and protein degradation. The frequently disturbed expressions of SR/hnRNP proteins in cancers lead to impaired functioning of target apoptotic genes, including regulators of the extrinsic (Fas, caspase-8, caspase-2, c-FLIP) and the intrinsic pathway (Apaf-1, caspase-9, ICAD), genes encoding Bcl-2 proteins, IAPs, and p53 tumor suppressor. Prototypical members of SR/hnRNP families, SRSF1 and hnRNP A1, promote synthesis of anti-apoptotic splice variants of Bcl-x and Mcl-1, which results in attenuation of programmed cell death in breast cancer and chronic myeloid leukemia. SR/hnRNP proteins significantly affect responses to chemotherapy, acting as mediators or modulators of drug-induced apoptosis. Aberrant expression of SRSF1 and hnRNP K can interfere with tumor responses to chemotherapy in pancreatic and liver cancers. Currently, a number of splicing factor inhibitors is being tested in pre-clinical and clinical trials.

In this review we discuss recent findings on the role of SR and hnRNP proteins in apoptotic control in cancer cells as well as their significance in anticancer treatments.

Introduction

Apoptosis is the process of controlled removal of cells that are damaged or unwanted at given developmental stage or physiological state [1,2]. In cancer, apoptotic control of cell quality and quantity is insufficient or even lost. Hence, the cells bearing molecular aberrations that allow for their uncontrolled proliferation are not eliminated and contribute to malignant transformation. Attenuation of apoptosis results also in resistance of tumor cells to therapeutics.

The mechanism of apoptosis is complex and involves two, distinct regulatory pathways: the death receptor-mediated (or extrinsic) pathway and the mitochondrial (or intrinsic) pathway (Fig. 1). The death receptor pathway is activated when death ligands, TRAIL (TNF-related apoptosis inducing ligand), Fas or TNF-α bind to death receptors (members of the TNF family) at plasma membrane. This leads to recruitment of adaptor proteins, TRADD or FADD and formation of death-inducing signaling complex (DISC) that subsequently activates initiator caspases 8 or 10. Activated initiator caspases cleave and activate downstream effector caspases 3, 7 and 6 that trigger the execution of apoptosis [3,4]. This process may be negatively regulated by CFLAR protein (CASP8 and FADD-like apoptosis regulator; also known as c-FLIP) that binds to FADD and/or caspase-8 and prevents formation of DISC and activation of caspase cascade [5]. The pathway involving mitochondria is activated by internal stimuli such as DNA damage, hypoxia or oxidative stress that ultimately lead to loss of integrity of mitochondrial outer membrane (MOM) and cytoplasmic release of cytochrome c. This in turn results in formation of a complex called apoptosome, composed of cytochrome c, Apaf-1 and caspase-9 that finally activates executioner caspase-3. The intrinsic pathway is controlled by proteins of Bcl-2 family that can act either as pro-apoptotic (e.g.

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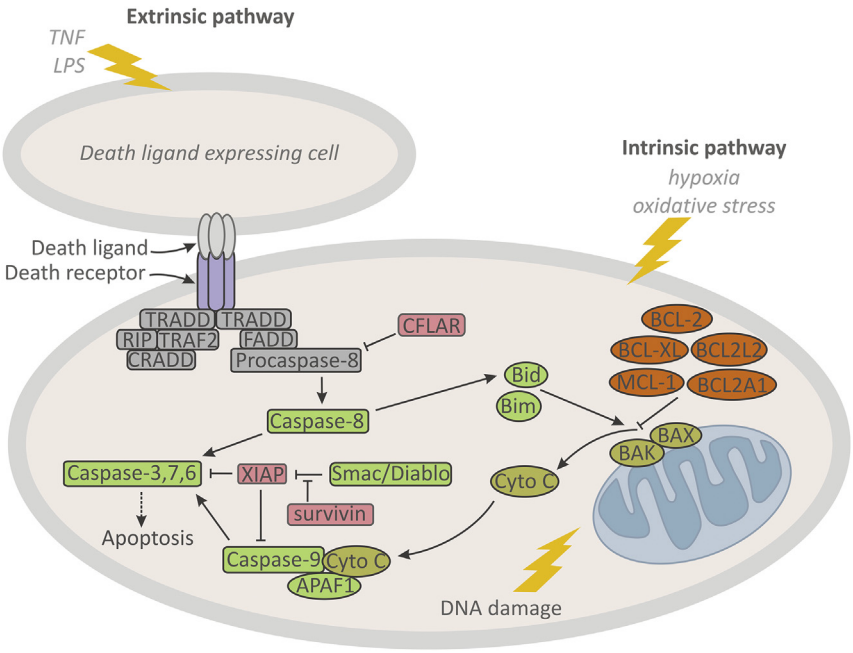

Fig. 1. The pathways of apoptosis. The extrinsic pathway (left): death ligands can be produced by cytotoxic lymphocytes in response to proinflammatory mediators (TNF, LPS) or by tumors that can induce apoptosis of immune cells. Death ligands interact with death receptors at plasma membrane, leading to recruitment of adaptors proteins (TRADD, FADD) and formation of death-inducing signaling complex (DISC) that subsequently activates initiator caspases (-8 or -10). Activated initiator caspases cleave and activate downstream effector caspases (-3, -7, -6) that trigger the execution of apoptosis. CFLAR is a negative regulator of apoptosis that binds to FADD and/or caspase-8 and prevents formation of DISC and activation of caspase cascade. The intrinsic pathway (right): DNA damage, hypoxia or oxidative stress lead to loss of integrity of mitochondrial outer membrane and cytoplasmic release of cytochrome c. This in turn results in formation of apoptosome composed of cytochrome c, Apaf-1 and caspase-9 and finally leads to activation of executioner caspase-3. The intrinsic pathway is controlled by proteins of Bcl-2 family that can act either as pro-apoptotic (e.g. Bax, Bak, Bid and Bim) or anti-apoptotic proteins (e.g. Bcl-xl and Mcl-1) and, respectively, promote or inhibit release of cytochrome c. XIAP, survivin: inhibitors of apoptosis belonging to IAP family. The activity of IAPs is counteracted by Smac/Diablo protein that enables release of caspase-3 and -9 from binding with IAPs.

Bax, Bak, Bid and Bim) or anti-apoptotic proteins (e.g. Bcl-xl and Mcl-1) and, respectively, promote or inhibit release of cytochrome c [6,7]. Bcl-2 proteins can also influence apoptosis initiated by death-receptors [8]. Activation of caspases may be counteracted by IAPs (Inhibitor of Apoptosis Proteins: XIAP, cIAP1, cIAP2, survivin) [9]. On the other hand, the activity of IAPs can be counteracted by Smac/Diablo protein that enables release of caspase 3 and 9 from binding with IAPs. At the final step of apoptosis, the executioner caspases activate cytoplasmic endonucleases (CAD/ICAD) and proteases leading to degradation of chromosomal DNA, chromatin condensation, cytoskeletal reorganization and disintegration of the cell into apoptotic bodies [3].

Splicing factors are proteins involved in regulation of alternative splicing, the process by which introns are removed from primary transcript while exons are selectively joined to give different mRNA isoforms. Remarkably, alternative splicing of genes involved in apoptotic regulation often results in the synthesis of oppositely acting protein variants that either activate or inhibit programmed cell death [10,11]. The two key families of splicing factors are serine/arginine (SR) proteins and heterogenous ribonuclear proteins (hnRNPs). Both protein families include multiple members, including twelve classical SR proteins [12] and thirty seven canonical hnRNPs [13]. Most SR proteins act as splicing activators: they bind pre-mRNA at exonic splicing enhancers (ESEs) and facilitate exon recognition by splicing regulatory complex called spliceosome, enabling exon inclusion. SR proteins often compete with splicing repressors, such as hnRNPs, whose binding to exonic or intronic splicing silencers (ESSs or ISSs) blocks the access of spliceosome elements and inhibits splice site selection. SR proteins antagonize hnRNPs action in a concentration dependent manner, thereby preventing exon skipping [13–15]. The activity of both families of splicing factors is regulated by reversible phosphorylation, mediated by protein kinases, belonging to the SRPK and CLK families as well as by kinases activated in different signaling pathway, such as MAPK, PI3K, Akt [16]. Phosphorylation of splicing factors affects their binding to targeted transcripts, interactions with other proteins, and intracellular localization [17,18].

Recently, several excellent reviews discussed the role of splicing factors in DNA damage [19] or carcinogenesis and anticancer therapy [20–22]. However, to our knowledge, the importance of both splicing factor families in cancerous deregulation of apoptosis was not summarized so far. In this review we focus on recent findings on the role of SR and hnRNP proteins in apoptotic control in cancer cells as well as their significance in anticancer treatments.

Apoptotic genes as targets of splicing factors

Cancer cells often avoid apoptosis by changing the expression of genes that control programmed cell death [23]. Changed expression of splicing factors, frequently observed in cancers (Table 1), can affect apoptosis in several ways. Too high or too low level of splicing factors can result in changed balance between pro- and anti-apoptotic splice variants. Splicing factors can also facilitate synthesis of specific splice variants that promote or interfere with apoptotic pathways. Furthermore, splicing factors can act as translational or posttranslational regulators by altering IRES-mediated translational rate or influencing proteasomal degradation of apoptotic proteins. Finally, splicing factors can also directly interact with apoptotic proteins and modulate their activity (Fig. 2). The examples of key apoptotic regulators and their regulation by splicing factors are discussed below.