

review

Survivin - an inhibitor of apoptosis and a new therapeutic target in cancer

Jože Pižem¹ and Andrej Cör²

¹Institute of Pathology and ²Institute for Histology and Embryology,
Medical Faculty, Ljubljana, Slovenia

Survivin is a unique member of the inhibitor of apoptosis (IAP) protein family. It inhibits apoptosis by interfering with post-mitochondrial events during apoptosis, thus blocking activation of caspases. The expression of survivin is among the most tumour specific of all human genes. It is overexpressed in most human cancers but is not detected in most normal tissues. Some molecular mechanisms of survivin upregulation in cancer have been elucidated, including loss of the wild-type p53. Tumours that overexpress survivin generally bear a worse prognosis and are associated with resistance to therapy. Its differential expression in cancer versus normal tissues makes survivin detection a useful tool in cancer diagnostics and a promising therapeutic target. Survivin targeting has resulted in increased spontaneous and induced apoptosis and inhibition of tumour growth. Some anticancer drugs currently introduced into clinical practice might well act by inactivating survivin.

Key words: apoptosis - drug effects; caspases; protein p53; neoplasms

Introduction

Apoptosis, programmed cell death, maintains the homeostasis in tissues by regulating the balance between cell proliferation and cell death. A diminished ability of cancer cells to undergo apoptosis has been recognised as an important mechanism of tumour growth and progression. Failure to undergo apoptosis in

the face of unrepaired damage leads to enhanced mutation, including chromosomal alterations, and can be a cause of the genomic instability that is a general characteristic of cancer progression.¹ Since the first description of apoptosis, three decades ago, as a special type of cell death with unique morphological characteristics, a complex genetic programme of cell suicide has been elucidated.^{2,3}

In mammalian cells, apoptosis can be triggered either by extrinsic or intrinsic pathways. An extrinsic pathway is initiated by ligation of death receptors on the cell surface (CD95/Fas receptor, tumour necrosis factor- α receptor) and acts through the activation of initiator caspase 8. An intrinsic pathway is

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Correspondence to: Jože Pižem, Institute of Pathology, Medical Faculty, Korytkova 2, 1000 Ljubljana, Slovenia. E-mail: jozepizem@hotmail.com

triggered by multiple death signals, either intracellular (unrepaired DNA damage) or environmental, that all culminate in dysregulation of the mitochondrial function. It results in an increased permeability of the outer mitochondrial membrane leading to release of mitochondrial proteins, including cytochrome c and SMAC/DIABLO. These proteins facilitate initiator caspase 9 activation, through a multi-protein complex called apoptosome (Figure 1).

Caspases, a family of cysteine proteases, are the key mediator molecules of apoptosis.⁴ They are present in the cell as inactive precursors (procaspases) and are activated by proteolytic cleavage. Caspases are either initiator caspases (caspase 8 and 9) or effector caspases (caspase 3 and 7). Initiator caspases are activated by self-processing in multimeric

protein complexes (such as apoptosome). Activated initiator caspases, in turn, cleave downstream effector caspases in a proteolytic cascade. Both intrinsic and extrinsic pathways converge to activate effector caspases. Finally, activated effector caspases specifically cleave cellular proteins that are involved in DNA repair, cytoskeletal organisation and nuclear integrity. This is the basis for morphological changes of apoptotic cells (nuclear fragmentation, condensation of the cytoplasm, detachment from the neighbouring cells, apoptotic bodies formation) and their clearance by phagocytosis.

Two gene families of apoptosis regulators have been identified - the Bcl2 family, and the inhibitor of apoptosis (IAP) family. Bcl2 proteins are thought to regulate mitochondrial

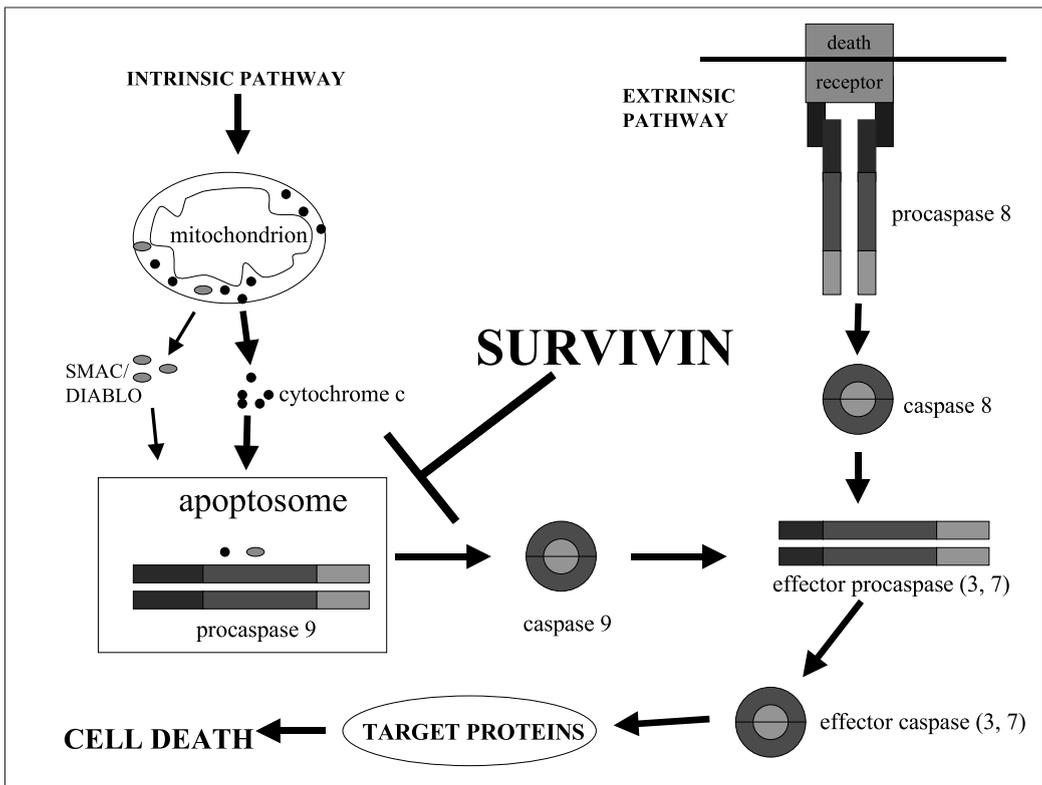


Figure 1. Apoptotic pathways and the site of survivin antiapoptotic action. Intrinsic and extrinsic apoptotic pathways are shown that converge into a common downstream pathway of effector caspase activation. Survivin most probably blocks, directly and/or indirectly, caspase 9 activation.

permeability by either decreasing or enhancing the release of mitochondrial proteins, particularly cytochrome c, therefore modulating the intrinsic pathway of apoptosis. In contrast, IAP proteins possess only an inhibitory function and act downstream by preventing activation of caspase 9 in apoptosome and inhibiting the activity of effector caspases.⁵

Survivin is a unique member of the IAP family. It is of special interest because it is overexpressed in most human cancers but is not detected in most normal tissues. This fact makes survivin a molecular marker of cancer and a promising cancer therapeutic target.

Survivin structure and its subcellular distribution

Human survivin is a 16.5 kDa intracellular protein that belongs to the inhibitor of apoptosis (IAP) gene family. In humans, eight members of the IAP family have been identified, including NAIP, XIAP, c-IAP1, c-IAP2, Ts-XIAP, ML-IAP, Apollon and survivin. IAPs are characterised by carrying 1-3 copies of a 70-amino-acid zinc-finger fold, which is designated the baculovirus IAP repeat (BIR).⁶ Survivin is the smallest IAP member, having a single BIR repeat and a homodimeric structure.⁷ It consists of 142 amino acids, its gene spans 14.5kb at the telomeric position of chromosome 17 and has four exons and three introns.⁸

A single copy of the survivin gene gives rise to three alternatively spliced transcripts. In addition to wild-type survivin, two survivin isoforms are generated by the insertion of an alternative exon 2 (survivin-2B) or removal of exon 3 (survivin-ΔEx-3).⁹ Because of the frameshift, the latter has a unique carboxyl terminus sequence containing a nuclear localisation signal, which is found exclusively in survivin-ΔEx-3 and may be implicated in subcellular targeting survivin to mitochondria and nucleus.^{9,10} While survivin

and survivin-ΔEx-3 are both antiapoptotic, survivin-2B has lost its antiapoptotic potential and might be a naturally occurring antagonist of antiapoptotic survivin variants.¹⁰

In mitosis, survivin has been shown to localise to various components of the mitotic apparatus, such as centrosomes and possibly microtubules. In tumour cells, the location of survivin is abnormal, with survivin present diffusely throughout the cytoplasm and often in the nucleus.^{6,11}

Survivin shows a clear cell-cycle dependent expression at mitosis. This is largely controlled at the transcriptional level and mediated by cell-cycle dependent elements and cell-cycle homology regions that are located in the proximal survivin promoter. These regions are typically found in genes expressed in the G2/M phase of the cell cycle, such as cyclins A and B. In synchronised HeLa cells, transcription of the survivin gene is increased in G2/M by more than 10-fold, as compared to G1 or S arrested cells.¹² Polyubiquitylation and proteasome-dependent degradation at interphase and mitotic phosphorylation leading to increased stability at metaphase contribute to survivin accumulation at mitosis. Especially in non-transformed cells (CD34+ bone-marrow-derived stem cells, endothelial cells), survivin is upregulated in response to cytokine stimulation in a cell-cycle independent manner.⁵ Endothelial cells upregulate their survivin expression after stimulation with vascular endothelial growth factor (VEGF).⁶

Survivin expression in normal and neoplastic tissues

Survivin expression has been extensively studied in neoplastic and non-neoplastic tissues by Western blotting, in situ hybridisation and immunohistochemistry. Survivin is strongly and diffusely expressed in embryonic and foetal organs, but is undetectable in most terminally differentiated normal tis-

sues. Adult normal cell types that express survivin include thymocytes, CD34+ bone-marrow-derived stem cells, endothelial cells, basal epithelial cells of colonic mucosa and epithelial cells of normal uterine cervix.^{5,13} Week signals have been detected in placenta and proliferative and secretory endometrium.^{8,14}

Notably, in contrast to normal tissues, survivin expression is dramatically upregulated in cancer, and survivin has been identified as the fourth transcriptome expressed in cancers of colon, lung, breast, brain and melanoma, but low or undetectable in the same normal organs.⁷ An analysis of a panel of 60 different cancer cell lines revealed ubiquitous expression of survivin in all cell types, but at different levels.¹²

The molecular mechanisms of survivin overexpression in cancer seem to be complex and are only partially understood. Given the widespread survivin expression in many types of cancer, it is very likely that multiple pathways are involved in the reactivation of the survivin gene. There is compelling evidence that survivin overexpression does not simply reflect the presence of a higher number of proliferating cells, as the percentage of survivin positive cells in a tumour typically exceeds the number of proliferating cells measured by Ki67 labelling.⁵

Several molecular mechanisms implicated in survivin overexpression in cancer have been elucidated. In neuroblastoma, a frequent genetic abnormality is amplification of 17q25, containing the survivin locus.¹⁵ Survivin exon 1 sequences are silenced by methylation in normal ovaries, but become unmethylated, and thus transcriptionally active in ovarian cancers.⁷ Recently, wild-type p53, but not mutant p53, was shown to repress survivin expression in various human cancer cell lines.^{16,17} A positive correlation between survivin expression and p53 accumulation (indicating its mutation) has been reported in gastric cancer¹⁸, pancreatic adenocarcinoma¹⁹,

but not in transitional cell carcinoma of the upper urinary tract²⁰ or colorectal carcinoma.²¹ Upregulation of survivin in colorectal cancer might result from APC (adenomatous polyposis coli) mutations.⁵

Survivin overexpression has been shown in preneoplastic lesions of the skin (actinic keratosis), pancreas (intraductal neoplasia), uterine cervix (intaepithelial neoplasia) and colonic adenomas, suggesting that survivin upregulation occurs early during tumourigenesis.^{5,12,13,22,23} In colorectal carcinogenesis, there is a significant increase of survivin positive cases in transition from normal mucosa to adenoma with low dysplasia and from adenoma with low dysplasia to adenoma with high dysplasia.^{24,25}

Three patterns of immunostaining to survivin are generally observed in tumour cells: 1. staining confined to the cytoplasm, 2. predominantly nuclear staining and 3. intense staining of mitotic figures (Figure 2). While early immunohistochemical studies reported the expression of survivin limited to the cytoplasm, subsequent studies also showed nuclear localisation of survivin. Differential sub-cellular localisation of survivin could reflect the presence of different survivin splice variants. Survivin and survivin-2B preferentially localise in the cytoplasm, whereas survivin-

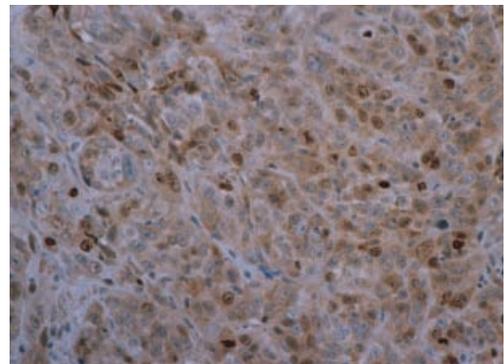


Figure 2. Immunostaining to survivin in hepatocellular carcinoma. Survivin is expressed in the cytoplasm and nucleus of tumour cells. Mitotic figures are intensely stained.

Δ Ex-3 localises in the nucleus.¹⁰ Nevertheless, results might be at least partially influenced by the use of different antibodies that recognise different epitopes. It is possible that the subcellular pools of survivin are immunohistochemically distinct, potentially reflecting post-translational modifications and/or epitope accessibility.⁵

Survivin function

The molecular mechanisms of survivin action are not fully elucidated and are, at least in some aspects, controversial. Nevertheless, it is a generally accepted view that survivin is an inhibitor of apoptosis and it interferes with cell-cycle progression and microtubule stability.

There is a huge body of experimental evidence characterising survivin as an inhibitor of apoptosis. In cell culture systems, overexpression of survivin has been consistently associated with inhibition of apoptosis initiated by both the extrinsic and intrinsic pathways.⁵ Survivin counteracts apoptosis induced by certain chemotherapeutic drugs.¹² Apoptosis, either spontaneous or induced, is suppressed in organs and tissues of transgenic animals that express survivin.⁵

In general, mammalian IAPs block apoptosis by direct or indirect inhibition of terminal effector caspase 3 and 7 and initiator caspase 9.^{6,12} There is no good evidence that survivin operates through direct contact with effector caspases. Recent studies suggest that survivin particularly inhibits the intrinsic pathway of apoptosis by interacting with post-mitochondrial events, as indicated by its ability to localize to mitochondria and to associate with caspase 9 and SMAC/DIABLO (Figure 1).^{5,8,26}

In addition to its role in inhibiting apoptosis, survivin localisation to the mitotic apparatus indicates its role in cell division. Targeting survivin has resulted in aberrant mitotic pro-

gression, leading to failed cytokinesis and multinucleation.⁵ Survivin is indispensable during embryonic development; its homozygous deletion in mice leads to inevitable lethality at day 4-5, due to defects in mitotic spindles formation.⁶ The apparent requirement for survivin in normal cell division suggests that overexpression of survivin in tumours could perturb normal cell cycle control.

Survivin as a diagnostic and prognostic marker of cancer

Its differential expression in cancer, compared to most normal tissues, makes survivin a candidate for a molecular marker of cancer. Generally, survivin is (over)expressed in the majority of cases within a certain tumour type, the percent of positive cases ranging typically from approximately 30% to 100%.¹² Moreover, in retrospective trials, high survivin expression in tumours has been associated with shortened overall survival, an increased rate of recurrence and resistance to radiation and chemotherapy.^{8,14,24,27,28} These data, in conjunction with a dual role of survivin in inhibiting apoptosis and promoting cell proliferation, indicate that survivin might confer growth and survival advantages for tumour onset and progression.⁵ Indeed, an association between survivin expression and diminished apoptotic rates and/or higher proliferation activity has been reported.^{14,24,27,28} There is good evidence that survivin is a powerful negative prognostic marker in most tumours studied. This fact might warrant a simple immunohistochemical detection of survivin in tumour specimens, which might provide a quick prognostic indicator for identifying patients at risk of recurrent disease and those who would benefit from more aggressive follow-up and alternative treatment protocols.⁷

Survivin can be detected in biological fluids of cancer patients, as a result of the shed-

ding of tumour cells from the primary sites. Survivin detection in the urine has proved to be a specific and sensitive marker of bladder cancer. Alternatively, circulating antisurvivin antibodies have been detected in cancer patients and detecting them could provide a potential diagnostic (screening) tool.⁷

Survivin as a therapeutic target

Survivin is an attractive therapeutic target because it is selectively expressed in cancer and is potentially required for the viability of cancer cells. A survivin-based anticancer therapy would be expected to carry limited toxicity for normal cells and to be effective in removing the general cell-viability system provided by survivin overexpression.²⁹

Various approaches to targeting survivin have been tested *in vitro* and *in vivo*. Survivin synthesis (translation from survivin mRNA) can be blocked by using antisense technology and ribozymes. With the use of these approaches, loss of survivin expression can be sufficient to trigger apoptosis, to enhance chemotherapy-induced and radiotherapy-induced apoptosis and to dysregulate cell proliferation in tumour but not in normal cells.^{5,30}

Experiments using phosphorylation defective survivin mutant Thr34→Ala revealed that phosphorylation of threonin at position 34 is required for cancer cell viability and might contribute to survivin stability at mitosis. Wild-type survivin is phosphorylated at Thr34 by mitotic kinase Cdc2-cyclin-B1. Survivin mutant Thr34→Ala functions as a dominant-negative mutant that competes with wild-type survivin, thereby blocking its activity. Adenoviral delivery of survivin Thr34→Ala suppressed tumour growth in cancer xenograft models *in vivo*.⁶

Survivin phosphorylation at Thr34 can be inhibited pharmacologically by recently developed antagonists of cyclin-dependent ki-

nases, such as flavopiridol, which blocks cyclin-dependent kinases, including Cdc2.⁵

Recent observations suggest that T cells can mount a cytolytic response against survivin peptides and HLA class I restricted cytolytic T cells exist in patients with different cancers *in vivo*. A cancer specific immune response to survivin might therefore be used for potential vaccination strategies.⁵

Survivin is expressed in endothelial cells during angiogenesis and is associated with resistance to apoptosis. Ablation of survivin during angiogenesis caused endothelial cell apoptosis and promoted involution of three-dimensional capillary-like vessels *in vitro*. Therefore, by survivin targeting, tumour growth suppression could be at least partially mediated by inhibition of tumour angiogenesis.^{7,31}

Conclusions

Survivin is an inhibitor of apoptosis and might be required for maintenance of cancer cell viability and cell-cycle progression. Its differential expression in cancer cells versus normal tissues has two important implications; it makes survivin a molecular marker of cancer and an attractive therapeutic target. Survivin is expressed in the majority of various cancers studied and, notably, it is generally considered a negative prognostic marker of cancer. Various strategies to targeting survivin in cancer cells are currently under investigation, giving promising results in *in vitro* and *in vivo* models. Moreover, some currently explored anticancer agents might mediate their anticancer effects by inhibiting the survivin pathway.

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