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Survivin – an anti-apoptosis protein: its biological roles and implications for cancer and beyond

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Summary

Survivin is a protein that inhibits apoptosis and regulates cell division. Survivin contains a **b**aculovirus **i**nhibitor of apoptosis **r**epeat (**BIR**) protein domain that classifies it as a member of the **i**nhibitor of **a**poptosis **p**rotein (**IAP**) family. Survivin inhibits apoptosis, via its BIR domain, by either directly or indirectly interfering with the function of caspases. Survivin is also a chromosomal passenger protein that is required for cell division. Survivin is expressed in embryonic tissues as well as in the majority of human cancers, but is not expressed in most normal adult tissues. The cancer-specific expression of survivin, coupled with its importance in inhibiting cell death and in regulating cell division, makes it a useful diagnostic marker of cancer and a potential target for cancer treatment. Recently, there is emerging evidence that survivin is involved in tissue injury and its healing. Understanding the mechanism of survivin function can potentially allow for the development of therapeutic strategies for cancer and other diseases.

key words: **survivin • apoptosis • cancer**

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WHAT IS SURVIVIN?

Survivin is a protein that inhibits apoptosis and regulates cell division [1]. Survivin was discovered in 1997 by hybridization screening of a human genomic library with the cDNA of the effector cell protease receptor-1 (EPR-1) [2]. The survivin gene spans 15 kb, and is located on chromosome 17 at band q25. Survivin has an unusual relationship to EPR-1 in that its sequence is complementary to and in the reverse orientation of EPR-1. The coding strand of survivin contains an open reading frame of 426 nucleotides, and encodes a protein of 142 amino acids, with a molecular weight of approximately 16.3 kDa [2].

Survivin over-expression *in vivo* increases cell resistance to apoptosis. Transgenic expression of survivin in epidermal keratinocytes significantly reduced the number of apoptotic cells in the epidermis following exposure to UV irradiation [3]. Conversely, inhibition of survivin expression *in vitro*, by antisense survivin oligonucleotide treatment, increased the susceptibility of Hela cells to receptor-mediated apoptosis [4] and induced apoptosis in the human neural tumor cell lines, MSN and TC620 [5].

Survivin appears to have an important role in regulating apoptosis at cell cycle checkpoint(s). Survivin expression is highly cell cycle-regulated, and is detectable in the nucleus selectively at the G2/M phase [6]. Transcription of survivin has been shown to be directly repressed by p53, another cell cycle checkpoint-regulating protein that induces apoptosis [7]. When acute lymphoblastic leukemia cells are treated with doxorubicin, which causes accumulation of wild type p53, the result is a dramatic down-regulation of survivin, depletion of cells in the G2/M phase of the cell cycle, and increased apoptosis [8].

Survivin appears to be important for cell cycle progression as well. Disruption of survivin by antisense targeting in Hela cells results in spontaneous apoptosis and aberrant mitosis [9], as well as an increase in caspase-3 activity at mitosis [6]. Disruption of survivin in cell lines by both antisense targeting and survivin antibodies also induces polyploidy and aneuploidy as a result of cytokinesis failure and the premature onset of anaphase [10]. *In vivo*, survivin is also required for cell division. Homozygous knockout of the survivin gene in mouse embryonic stem cells results in disrupted microtubule formation and polyploidy during development, which culminates in early embryonic lethality [11].

PROTEIN STRUCTURE OF SURVIVIN

The structure of the survivin protein is intimately linked with its function as an inhibitor of apoptosis. The amino terminal portion of survivin consists of three alpha helices (residues 14-21, 31-41, 68-80) and 3 beta-sheets (residues 43-45, 55-58, 61-64), which closely resemble the BIR domain that is conserved in the IAP family (Fig. 1) [2,12]. The BIR domains of IAP family members are involved in the function of these proteins as inhibitors of apoptosis [13]. A mutation in the BIR domain, T34A, which inhibits phosphorylation of survivin by $p34^{cdc}$ – cyclin B1, abrogates the ability of survivin to inhibit apoptosis [14].

The role of survivin in cell division is also suggested by its protein structure. Survivin closely resembles the BIR-containing proteins from yeast and*C. elegans*, which are involved in cytokinesis [13,15]. The BIR domain of survivin is followed by a long α-helical region [6,12], as illustrated in Figure 1. Removal of this c-terminal helix prevented survivin localization to microtubules [6].

Crystal structure analysis of survivin revealed that it exists as a dimer, with the two BIR domains forming a 'bow-tie' shape [12]. The c-terminal helix is not involved in the dimerization of survivin but extends outward from the entwined BIR structure [12]. How the dimeric structure of survivin is involved in inhibition of apoptosis and cytokinesis requires further investigation.

ROLE OF SURVIVIN IN INHIBITION OF APOPTOSIS

Survivin plays an important role in the suppression of apoptosis by either directly or indirectly inhibiting the activity of caspases, the cell death proteases that induce apoptosis. Several IAP family members have been shown to suppress apoptosis by direct inhibition of caspases [16] via the BIR domains [13]. The structure of survivin has been compared to another IAP family member, XIAP, which contains three BIR domains. XIAP inhibits caspase-3 and caspase-7 via a linker region between the first two BIR domains, and also binds to and inhibits caspase-9 through its third BIR (BIR3) domain [17]. The BIR domain of survivin appears closely related in three-dimensional structure to the BIR3 domain of XIAP, suggesting the possibility that survivin binds caspase-9 [18]. The interaction between survivin and caspase-9, and the functional implications of this interaction, have been studied through mutagenesis. Loss of phosphorylation at threonine 34 on the T34A mutant of survivin results in dissociation of an immunoprecipitable survivin-caspase-9 complex on the mitotic apparatus, allowing caspase-9 dependent apoptosis to occur [14].

Figure 1. Representation of survivin protein structure. Survivin is a 142 amino acid protein containing a BIR domain (black bar) at the amino terminal region and a long alpha-helical region (striped bar) at the carboxyl terminus.

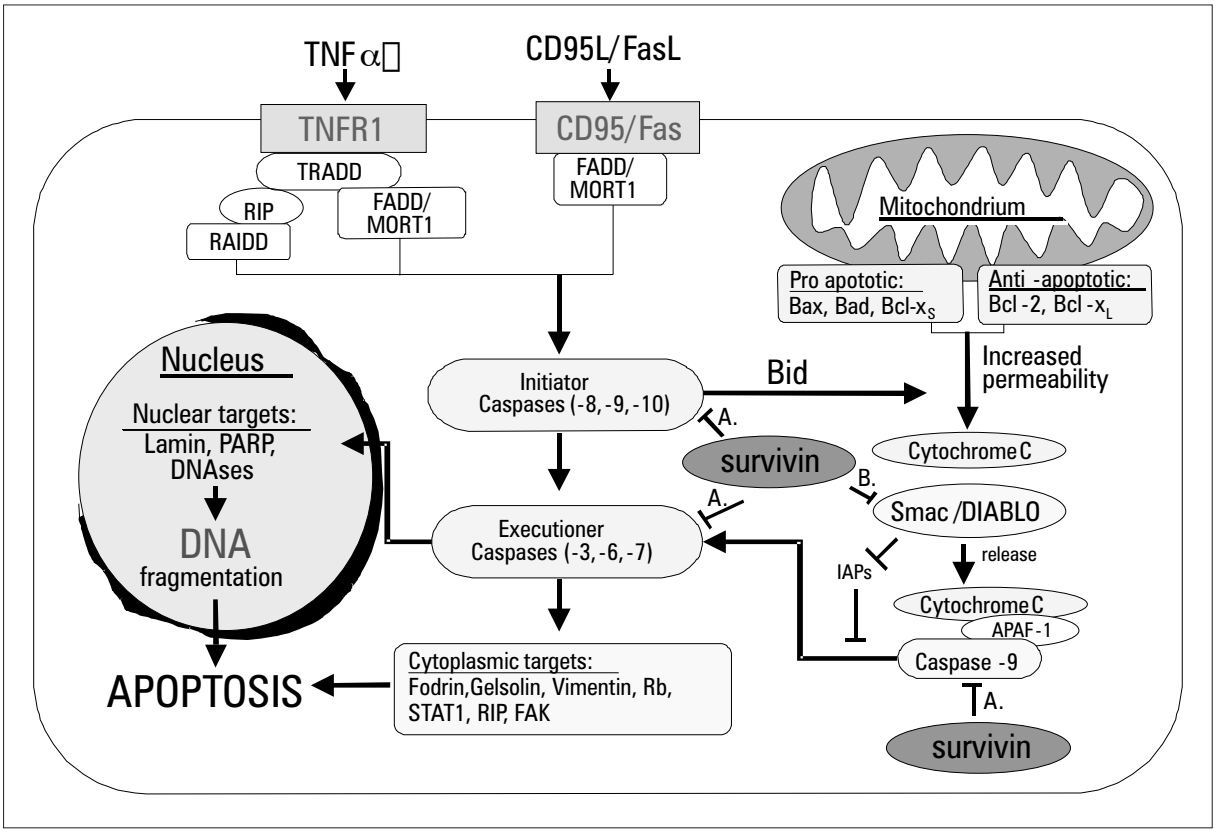

Figure 2. The role of survivin in apoptosis pathways. Survivin (green) may regulate apoptosis by: A. Directly inhibiting the caspases responsible for induction and execution of apoptosis. B. Indirectly inhibiting caspase function by regulating Smac/Diablo.

Direct binding between survivin and the caspases has not been confirmed, but survivin may also inhibit caspase activity indirectly. There is evidence of an indirect regulation of caspase activity by survivin in the mitochondrial pathway of apoptosis. Hepatocytes of heterozygous survivin knockout mice contain low basal levels of activated procaspase-8, Bid, procaspase-9, and procaspase-3, and have increased susceptibility to Fas-induced apoptosis [19]. Fas-induced apoptosis is associated with release of cytochrome c and up-regulation of survivin in the mitochondria as well as in the nucleus and cytosol [19]. Upon Fas stimulation in cell culture, survivin has been shown to interact with Cdk4, which releases p21 from its complex with Cdk4 [20] making it possible for p21 to complex with caspase-3, the initial step in inactivating caspase-3 in the mitochondria. Also, ectopic overexpression of a second mitochondrial-derived activator of caspases, (smac)/DIABLO, increases Apo-2L/TRAIL-induced caspase-3 activity, and down-regulates the activity of survivin and other IAPs such as XIAP and cIAP1 [21]. Smac/Diablo is released from mitochondria into the cytosol along with cytochrome c during execution of the mitochondrial apoptosis pathway. Smac/DIABLO can promote apoptosis by binding to and suppressing the inhibitory effects of the IAP proteins [22]. Survivin may possibly function to inhibit caspase activity indirectly via binding to and sequestering Smac/DIABLO, thus preventing Smac/DIABLO binding to other IAPs (Fig. 2, [23]).

The mechanism of caspase-3 inhibition by survivin remains controversial. There are reports indicating that purified survivin directly binds to caspase-3 and inhibits its activity *in vitro* [14,24,25]. However, the survivin protein lacks the linker region found in other IAP members that is responsible for their interaction with caspase-3 [17]. If there is a unique mechanism by which survivin directly inhibits caspase-3 activity, it has yet to be shown. Further investigation is required to determine how survivin inhibits apoptosis through suppression of caspase-3 and other caspases.

ROLE OF SURVIVIN IN CELL DIVISION

Recent reports demonstrate how survivin may regulate cell division. During the cell cycle, survivin is first detected on centromeres at prophase/metaphase. It is present in the spindle midzone during anaphase/telophase, but is no longer detected by the end of telophase [6,11]. Another report has indicated that survivin is localized to kinetochores until metaphase, then to the spindle midzone in anaphase and in the cleavage plane during telophase and cytokinesis [26]. These localization patterns resemble those of the inner centromere protein (INCENP) [11], TD-60 [27], and Aurora B [28], which are known as chromosomal passenger proteins. Thus, based on its localization during the cell cycle, survivin is postulated to be an additional chromosomal passenger protein. These four proteins

are the only mammalian passenger proteins known to date. Chromosomal passenger proteins are carried on the chromosomes to the center of the cell at metaphase, in the plane of the future cleavage furrow, and are important for cytokinesis and chromosome movement during cell division.

Disruption of survivin results in cell division defects. Survivin (–/+) mouse embryos are polyploid, show disrupted microtubule formation, and are remarkably similar to those of INCENP knockout mice [11]. This suggests that survivin and INCENP function in the same pathway(s) during cell cycle progression. Indeed, INCENP has been shown to bind survivin and to target it to the centromeres and mitotic spindle [29]. Survivin is also reported to bind Aurora-B and to enhance the phosphorylating activity of Aurora-B toward its substrates, such as histone H3 [30]. Aurora-B is a mitotic serine/ threonine kinase that plays an important role in chromosome segregation and cytokinesis [31]. Thus, survivin functions jointly with other passenger proteins such as INCENP and Aurora-B to regulate cell division.

SURVIVIN EXPRESSION AND GASTROINTESTINAL CANCERS

Recently, there has been great interest in survivin as a diagnostic marker and potential drug target because of its predominantly cancer-specific expression in adult human organ tissues. Survivin is expressed in most human cancers including that of colon, breast, lung, pancreas, prostate, stomach, esophagus, and in high-grade non-Hodgkin's lymphoma [2,32,33]. During human development, survivin is expressed in fetal lung, heart, liver, kidney, and gastrointestinal tract, and in fetal tissues where apoptosis occurs, such as the stem cell layer of stratified epithelia, endocrine pancreas and thymic medulla [34]. In all of these studies, survivin was not found in normal, adult tissues. These findings suggest that the cell division and anti-apoptosis functions of survivin are important not only during early development, but also during cancer progression as well.

In the gastrointestinal tract, there are indications that activation of survivin may be required for carcinogenesis. Yu et al showed that survivin expression is frequent (68%) in gastric cancer tissues and is also present, albeit at lower frequency (27%), in gastric mucosa of non-cancer relatives [33]. Survivin expression was also found in 22% of the non-cancerous tissues adjacent to gastric cancer tissue, but was absent in all of the normal, non-relative and non-adjacent gastric mucosal tissues that the authors examined [33]. In the esophagus, survivin expression has been shown to be associated with a high risk for cancer recurrence [32]. In the colon, survivin expression is detected in all normal colonic mucosa taken from noninvolved margins of carcinomas as well as the hyperplastic polyps and the adenomatous polyps [35]. During colorectal tumorigenesis, survivin protein expression is significantly and progressively increased during the transition from low dysplasia adenoma to high dysplasia carcinoma [36]. Thus, survivin expression is found in cancers, as well as in normal adult tissues that are predisposed to malignancy, indicating that survivin function may be required for carcinogenesis itself.

The role of survivin in the inhibition of apoptosis in gastrointestinal cancer has not been clearly established. Only one report demonstrated, by immunohistochemistry, that survivin-expressing gastric cancers have a significantly lower index of apoptosis when compared to survivin-negative cancers [37]. Another report examined the relationship between survivin expression in gastric cancer and DNA fragmentation (indicative of apoptosis) and found that survivin expression does not correlate with this hallmark of apoptosis [38]. The survivin pathway is possibly not entirely responsible for the inhibition of apoptosis in gastric cancers. Further investigation of survivin and other apoptosis inhibitors during tumor growth and progression may yield important insights into their functional role(s) in carcinogenesis and allow for the development of important therapeutic strategies for combating cancer.

SURVIVIN EXPRESSION IN NORMAL TISSUE

Numerous reports have examined the role of survivin in cancer, but little has been published about the role of survivin in normal tissue. Although, as previously mentioned, survivin expression was not found in most normal adult human tissues when examined for reports investigating the role of survivin in cancer, human survivin expression has been reported in some normal adult human tissues, including colonic mucosa [35], placenta [39], bone marrow [1] and keratinocytes of the basal layer of the skin [40]. Using the mouse model, expression of survivin was found to be elevated at the G2/M phase of the cell cycle during liver regeneration, and over-expression of survivin in a normal mouse liver cell line increases cell proliferation [41]. Survivin is also reported to play a role in vascular injury [42]. Specifically, up-regulation of survivin by PDGF stimulation in smooth muscle cells (SMC) promotes cell viability while disruption of survivin function in SMC prevents neointimal formation in mouse femoral arteries after injury [42]. Changes in cell death or viability in SMCs have been implicated in diseases of vascular remodeling [43]. Regulation of apoptosis constitutes an important medical problem, as it is crucial in combating conditions such as damage to kidney cells caused by ischemia-perfusion [44] and neuronal apoptosis in Alzheimer's disease [45]. Further investigation into the role of survivin in normal tissues may yield important further insights into the role of apoptosis in various disease states, and may possibly allow for the design of therapeutic strategies for the treatment of non-cancerous diseases.

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