Survivin: Role in Normal Cells and in Pathological Conditions

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Abstract: Survivin, an inhibitor of apoptosis (IAP) containing one baculovirus IAP repeat (BIR) domain, has been reported to be capable of regulating both cellular proliferation and apoptotic cell death. Survivin splice variants, survivin-ΔEx3 and survivin-2B, have apparently retained and lost anti-apoptotic potential, respectively. As survivin was first discovered due to its high homology with effector cell protease receptor (EPR-1), a protein involved in blood coagulation, it has been suggested (but not proven) that EPR-1 may act as a natural anti-sense to survivin in cells. Survivin homologs have been found in non-human species. Survivin expression has been described during embryonic development and in adult cancerous tissues, with greatly reduced expression in adult normal differentiated tissues, particularly if their proliferation index is low. Survivin has been defined as a universal tumor antigen and as the fourth most significant transcriptosome expressed in human tumors. Although survivin is usually located in the cell cytoplasmic region and associated with poor prognosis in cancer, nuclear localisation, indicative of favorable prognosis, has also been reported. Survivin expression has also been reported in a number of proliferating normal adult tissues.

Extensive research has been conducted, aimed at increasing our understanding of survivin, by determining its sub-cellular structure and location, mechanism(s) of action and control of expression. While much important information on this molecule has been accumulated, there are still many areas of controversy or limited information. Further research may enable exploitation of survivin overexpression in cancer compared to normal tissues, making survivin a potentially attractive target for cancer therapeutics.

INTRODUCTION

Apoptosis and Inhibitors of Apoptosis

Apoptosis

Apoptosis, or programmed cell death, is defined as a process of cellular suicide that proceeds with characteristic biochemical and cytological features, including condensation and fragmentation, accompanied by extensive blebbing of the cytoplasmic membrane. The dying cell is converted to membrane-bound vesicles called apoptotic bodies, which are rapidly phagocytosed [1]. Apoptosis is a physiological cell death process that enables metazoans to control their cell numbers and to eliminate unneeded senescent cells that may threaten the animal's survival (see reviews: [2-5]). The correct balance between apoptosis and inhibition of apoptosis preserves normal homeostasis and organ morphogenesis [6-7]. However, aberrations of this process underlie some human diseases, with abnormal inhibition of apoptosis involved in cancer [8-9] and autoimmune diseases, while excessive activation of apoptosis is implicated in neurodegenerative disorders such as Alzheimer's disease [10]. Members of the bcl-2 family of intracellular proteins are proposed to be regulators of activity of caspases (*i.e.* the protease enzymes involved in apoptosis). The opposing actions of anti- and pro-apoptotic members of this family play an important role in arbitrating the life-or-death decisions in cells [11].

Inhibitors of Apoptosis

Inhibitors of apoptosis (IAPs) are a family of evolutionarily conserved molecules found in many species. 9 mammalian IAPs have been identified. The main distinguishing feature of all of the family members is a domain termed a baculovirus IAP repeat (BIR). Two types of BIRs exist, and IAPs have also been identified that contain single or multiple BIRs. It is proposed that the BIR domain is required for anti-apoptotic activity. However, as described below, the precise mechanism of action involved is yet to be determined.

During the last decade an evolutionarily conserved family of molecules regulating apoptosis (distinct from the Bcl-2 family), and termed inhibitors of apoptosis (IAPs) have been described. In 1993, the first member (termed IAP) of the IAP family was described in baculovirus, where it prevented the host suicidal response to infection [12]. The first report of a cellular IAP, *i.e.* NIAP, was made in 1994. NIAP inhibits apoptosis in nerve cells. Since then a number of

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IAPs have been identified in viruses, yeast [13-14], worms [15], flies [16] and mammalian cells [17-18]. The identifying feature of IAPs is the presence of at least one approximately 70 amino-acid zinc-finger module, designated baculovirus IAP repeat (BIR). Some IAPs also contain a C-terminal RING (C_3HC_4) finger, but the requirement of this RING finger for anti-apoptotic activity differs depending on the IAP and/or the nature of the apoptotic stimulus [19-24]. Other structural features found in some IAPs include a caspase-recruiting domain (CARD), an ubiquitin-conjugating domain, and a nucleotide-binding P-loop [24].

Two types of BIR domain exist [18]. The main structural difference identified between type I and type II domains is the conserved sequence in the BIR domain-encoding region of type II BIRs that is not present in type I BIRs, resulting in the type II BIRs being slightly longer than the type I BIRs. Type I BIRs are found in the following mammalian IAPs: X-IAP, cIAP-1, cIAP-2, ML-IAP (also termed livin) and NIAP; whereas type II BIRs occur in survivin and BRUCE, the insect IAPs, deterin and D-Bruce, the yeast IAP, SpBIR1P and ScBIR1P and nematode IAPs, CeBIR-1 and CeBIR-2. BIR domains can be further subgrouped into several sub-types and are characterised by a number of invariant amino acids. The three BIR domains – BIR-1, BIR-2 and BIR-3 – of human X-IAP, c-IAP1 and c-IAP2 are classified into 3 different subgroups, suggesting gene duplication of an ancestral IAP gene encoding 3 BIR domains [18]. Some, but not all BIR-containing proteins (BIRPs) are suppressors of cell death (*i.e.* anti-apoptotic), acting downstream of anti-apoptotic bcl-2 proteins which, *inter alia,* preserve mitochondrial integrity by inhibiting cytochrome c release. Apparently, at least one BIR motif is essential for anti-apoptotic activity [23, 25], with some IAP family members containing up to 3 tandemly repeated BIRs (Fig. (**1**)).

Solution structure analysis shows that a BIR folds into a structure typically comprising a series of 4 or 5 α helices and three-stranded β sheets [18] with a highly hydrophobic center that includes a C_2HC motif coordinating a zinc ion. This core structure includes a number of highly conserved cysteine and histidine residues, which are required for anti-apoptotic activity [18, 22, 26]. The mechanisms of action of the IAPs may involve a physical association with initiator and effector caspases, preventing the proteolytic maturation and enzyme activity of the caspases (Smac, a mitochondrial protein, apparently inhibits such IAP-dependent cell protection by binding to the IAP and releasing the caspase for apoptosis) [24, 27]. As a number of IAPs interact with caspases through BIR-containing regions, it is assumed that BIRs are caspase-interacting regions. The reason why there are multiple BIR domains in some, but not other IAPs, is not clear. BIRs have been identified and characterised in *C. elegans*, namely BIR-1 and BIR-2 [28]. BIR-1 was found to be highly expressed during embryogenesis and was also detectable during other stages of development, whereas BIR-2 was only expressed in embryos and adults. Embryos lacking BIR-1 were unable to complete cytokinesis and became multinucleated. Inhibition of BIR-1 did not increase

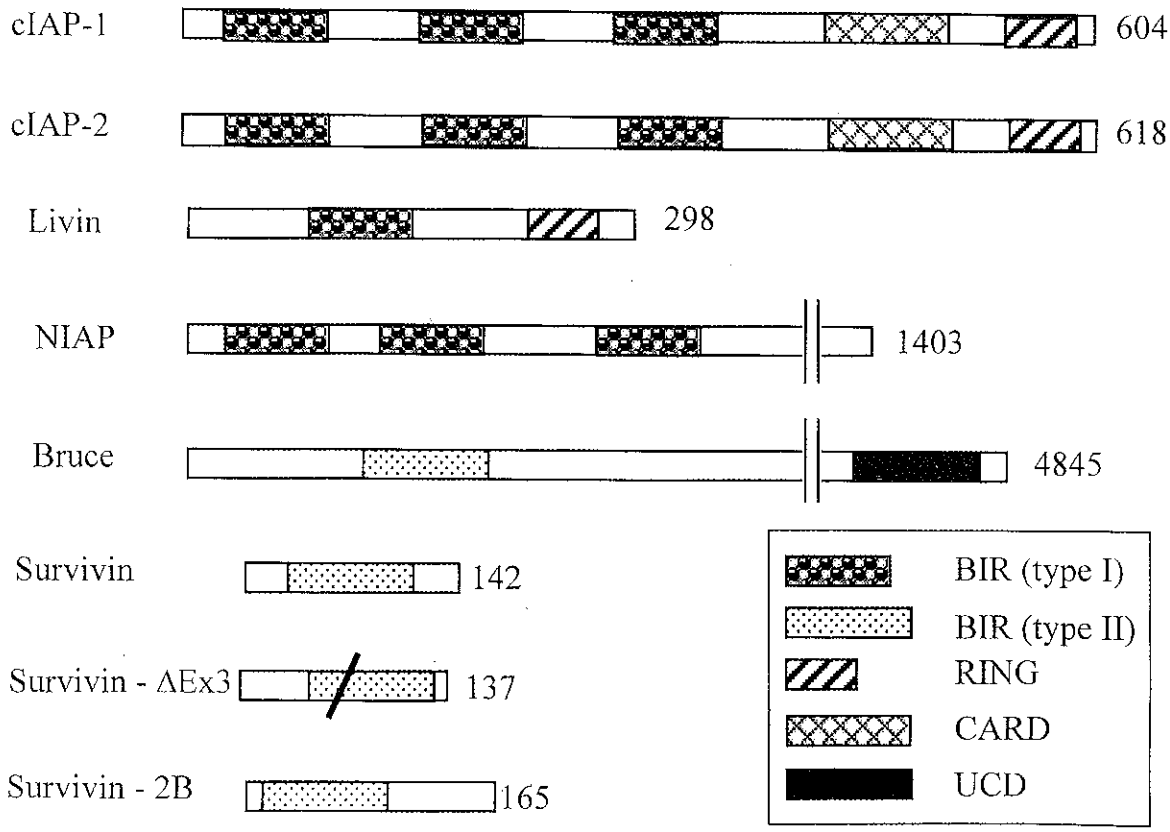

Fig. (1). Domain structure of a range of mammalian IAPs. The number of amino acids in each IAP is indicated. Some mammalian IAPs contain type I BIRs, whereas other contain slightly longer type II BIRs. In survivin-ΔEx3, deletion of exon 3 (*I*) results in a frame shift with a slightly truncated BIR domain (see "The splice variants of Survivin"). Other features of some IAPs include a RING finger, a caspase-recruiting domain (CARD), and a ubiquitin-conjugating domain (UCD).

cell death and overexpression of BIR-1 did not inhibit cell death. Although both BIRs are required for full anti-apoptotic activity of the baculovirus IAP, Op-IAP, minimal anti-apoptotic activity is associated with the C-terminal BIR motif of Op-IAP, and the middle BIR (BIR-2) of X-IAP and survivin has only a single BIR domain [23]. In yeasts containing no caspases, gene knockout of BIR-domain containing proteins (BIRPs) results in defective meiosis and mitosis [13]. These results suggest that BIRs have evolved to acquire divergent biological roles [29].

Mammalian IAPs

A number of mammalian IAP-family members have been identified to date, including NIAP (neuronal apoptosis inhibitor) [30], c-IAP1 (cellular IAP 1), c-IAP2, X-IAP (X chromosome-linked inhibitor of apoptosis) [31], apollon, Ts-XIAP, livin [32-34], survivin and Bruce (see Fig. (**1**)). The spectrum of apoptotic stimuli that are blocked by mammalian IAPs is broad and includes ligands and transducers of the TNF family of receptors [35], Fas ligand [36-37], viral infection [38], chemotherapy agents [24, 39], exposure to UV radiation [36], pro-apoptotic members of the bcl-2 family, serum withdrawal [40], and over-expression of caspase family proteins [38, 41]. Of the mammalian IAPs described to date, X-IAP is believed to have the broadest and strongest anti-apoptotic activity. X-IAP, c-IAP1 and c-IAP2 have been shown to bind to specific cell death proteases, *i.e.* caspases-3 and 7 and procaspase-9 (but not caspase-1, -6, -8 or –10), and to inhibit their proteolytic activity *in vitro* [24, 42-44]. X-IAP binds and inhibits caspase-9 through BIR3 and it suppresses caspase-3 and -7 through a linker region located between BIR-1 and BIR-2 [45]. Like bcl family members that are involved in nearly all aspects of cell death/survival [11], IAPs may function in more than one way. In studies investigating inhibitors of apoptosis, it has been reported that IAPs can apparently arrest apoptosis before the caspases are involved [23].

SURVIVIN

Survivin is a mammalian IAP that is described as capable of regulating both cell proliferation and apoptotic cell death. Survivin expression has generally been described in fetal development and cancer, with relatively little expression in normal adult tissues. However, expression of survivin in normal cells and tissues (particularly those with high apoptotic indices) has also been reported. Identified due to its high homology to EPR-1, survivin has been mapped to chromosome 17q25 and consists of 4 exons and 3 introns. The resulting 1.9 kb message encodes a 16.5 kDa (142 amino acid) protein. Survivin contains a single BIR domain. As described below, structural studies suggest that survivin may exist as a homodimer.

The survivin gene, mRNA and protein have been studied extensively. Initial studies revealed strong survivin expression in several fetal tissues, whereas little or no survivin transcripts were detected in a range of normal adult tissues [17, 27, 46-48]. Furthermore, survivin protein was found to be expressed in the cytoplasmic region of most common human cancer cell types, but generally not in adjacent normal tissues. The indication of a correlation between expression of survivin and an unfavorable outcome in cancer suggested that survivin might be a potential prognostic factor and a tumor-specific target for cancer therapy. The discovery of survivin splice variants and of expression in some adult tissues as well as in tumors, different sub-cellular locations, and putative mechanisms of action, have somewhat complicated the situation. This review will focus on survivin, including its discovery, molecular organisation, varying isoforms, homologies between species, sub-cellular localisation, proposed cellular mechanism(s) of action, expression in normal and cancer cells, regulation of its expression, and how we may benefit from this knowledge in the future.

The Discovery of Survivin

Survivin was first described by Ambrosini *et al.* in 1997 [49], while hybridisation screening a human P1 genomic library with cDNA encoding a 65 kDa (337 amino acid) protein termed effector cell protease receptor-1 (EPR-1). EPR-1 is involved in blood coagulation and deposition of insoluble fibrin associated with cellular inflammatory responses and vascular injury, through interaction with factor Xa [50-51]. Survivin, the newly identified gene, was reported to be highly complementary to EPR-1 *i.e.* containing an open reading frame of 426 nucleotides complementary to EPR-1 [49]. More recently it was established that survivin and EPR-1 are encoded by structurally and topographically distinct messages (1.9 kb and 1.3 kb, respectively) originating from separate genes, belonging to a gene cluster within a contiguous physical interval of 75-130 kb on chromosome 17q25 [52].

Due to their complementary sequences, it has been suggested that there may be functional interactions between survivin and EPR-1 transcripts and/or their proteins and that EPR-1 may act as a natural anti-sense to survivin [49]. In support of this hypothesis, Ambrosini *et al.* [52] reported that transfection of Hela cells with EPR-1 cDNA resulted in a suppressed expression of endogenous survivin and loss of cell viability, due to increased apoptosis. However, results from analysis of survivin and EPR-1 expression in hematological malignancies do not conclusively indicate whether or not EPR-1 acts as a natural anti-sense to survivin *in vivo* [53].

The Molecular Organisation and Structure of Survivin

The human survivin gene is located on chromosome 17, localised to band q25, spanning 14.7 kb, and is approximately 3 % of the distance from the telomere [52]. This 14,796-nucleotide sequence encodes a 16.5 kDa (142 amino acids) intracellular protein containing a single BIR sequence (located in the N-terminal half) and a 3-amino acid insertion of Cys-Pro-Thr separating two hypothetical halves of the type II BIR domain [17]. The survivin gene comprises 4 exons and 3 introns and a TATA-less proximal promoter with an approximately 200-250 nucleotide canonical CpG region upstream of exon 1 [54].

The N-terminal Zn^{2+}-binding BIR domain of survivin consists of a three-stranded anti-parallel β sheet surrounded by 4 small α-helices [55]. The survivin sequence also contains a C-terminal 42 amino acid highly charged region predicted to form a coiled-coil, but suggested by other researchers to exist as an elongated C-terminal helix [55, 57]. Unlike some other IAPs, however, survivin does not contain a –COOH terminus RING finger or a CARD. The survivin sequence contains three putative protein kinase C (PKC) phosphorylation sites, two putative casein kinase II sites, and one putative protein kinase A (PKA) site, that may be of significance in survivin protein stabilisation and half-life (described as 2 hours by Muenchen *et al.* [56]) and regulation of its functional activities within the cell [17].

The survivin structure apparently consists of three separate, chemically distinct surfaces: one comprising many acidic residues, one containing basic residues, and another region rich in hydrophobic residues [57]. The acidic and basic patches are on the BIR domain, while the hydrophobic helical surface is associated with the C-terminal [55]. The crystal structure of survivin has recently been described comprehensively [55, 58, 59], so this topic will not be covered in detail in this review. In summary, using X-ray crystallography, all three research groups have described the survivin structure as a homodimer [57]; both solution studies and crystal analysis reveal a dimeric arrangement of survivin monomers consistent with a functionally relevant interaction surface. Two dimeric models have been proposed *i.e.* a bow-tie model and a zinc chelation model. The bow-tie model proposed by Chantalat *et al.* [59] is based on hydrophilic interactions between predominantly hydrophobic interfaces on the BIR domain of each survivin monomer in the dimer structure, whereas the zinc chelation model proposed by Muchmore *et al.* [58] relies on a bridging zinc atom mediating the dimeric interface. Although it was initially proposed that the 42 highly changed amino acids in the C-terminal region may form a coiled-coil [26] and contribute to the proposed dimeric interactions, it has more recently been suggested that the C-terminal domain forms a long and extended helix and that it is not involved in the dimer formation. In support of this, trypsin cleavage of survivin at residue 115 indicates that the N-terminal region is adequate for dimerisation [59]. Chantalat *et al.* [59] proposed that the C-terminal helices contain hydrophobic clusters with the potential for protein-protein interactions and that this may be the mechanism whereby survivin docks on microtubules.

Although two different models of survivin dimerisation have been proposed (possibly due to different protein purification and crystallisation procedures), it is apparent that interference with dimer formation as well as, for example, disruption of the acidic surface surrounding Asp 71 or inhibition of T^{34} phosphorylation (see "The subcellular localisation of Survivin" section) results in aberrant mitosis and cell death. This emphasises the significance of survivin dimerisation. Further determination of the survivin dimeric structure may enable us to understand its normal existence in cells and to target and selectively inhibit survivin.

The Splice Variants of Survivin

Apart from the main survivin protein, two splice variants, termed survivin-ΔEx3 and survivin-2B, have been described. Survivin-ΔEx3 encodes a protein which is smaller than survivin but apparently retains anti-apoptotic activity; survivin-2B is a larger protein with reduced anti-apoptotic potential. In cancer cell lines and tissues, survivin is generally described as the predominant survivin protein. Survivin-2B levels are

SURVIVIN: Accession No. AAC51660; Version AAC51660.1 GI:2315863

EXON 1	EXON 2	EXON 3	EXON 4

Protein sequence (142 amino acids; 16.5 kDa):
MGAPTLPPAW QPFLKDHRIS TFKNWPFLEG CACTPERMAE AGFIHCPTEN EPDLAQCFFC FKELEGWEPD DDPIEEHKKH SSGCAFLSVK KQFEELTLGE FLKLDRERAK NKIAKETNNK KKEFEETAKK VRRAIEQLAA MD

SURVIVIN-ΔEx3:

EXON 1	EXON 2	EXON 4

Protein sequence (137 amino acids; 15.7 kDa):
MGAPTLPPAW QPFLKDHRIS TFKNWPFLEG CACTPERMAE AGFIHCPTEN EPDLAQCFFC FKELEGWEPD DDPMQRKPTI RRKNLRKLRR KCAVPSSSWL PWIAESGRSC LVPEWLHHFQ GLFPGATSLP VGPLAMS

SURVIVIN-2B: Accession No. BAA93676; Version BAA93676.1 GI:7416053

EXON 1	EXON 2	EXON 2B	EXON 3	EXON 4

Protein sequence (165 amino acids; 18.6 kDa):
MGAPTLPPAW QPFLKDHRIS TFKNWPFLEG CACTPERMAE AGFIHCPTEN EPDLAQCFFC FKELEGWEPD DDPIGPGTVA YACNTSTLGG RGGRITREEH KKHSSGCAFL SVKKQFEELT LGEFLKLDRE RAKNKIAKET NNKKKEFEET AKKVRRAIEQ LAAMD

Fig. (2). Human survivin splice variants. Underlined amino acids indicate those hat differ in survivin-ΔEx3 and survivin-2B (due to deletion of exon 3 and inclusion of exon 2B, respectively) compared to survivin.

reduced in some cases of tumor progression and this splice variant has been proposed to act as a dominant-negative to survivin.

Mahotka *et al.* [60] reported the discovery of two novel alternatively processed survivin transcripts (see Fig. (**2**)). These splice variants are designated survivin-ΔEx3 and survivin-2B, based on their modified transcript structures. Survivin-ΔEx3 lacks the 118 nucleotide long exon 3, resulting in a protein product of 15.7 kDa (137 amino acids), compared to the 16.5 kDa (142 amino acids) survivin protein. Survivin-2B retains part (69 nucleotides *i.e.* 23 amino acids) of intron 2 as a cryptic exon, designated exon 2B. Survivin-2B encodes an 18.6 kDa (165 amino acid) protein. The sequence alterations involved in the survivin-ΔEx3 and survivin-2B transcripts result not only in proteins of different sizes to survivin, but they also cause structural changes in domains of the resulting proteins. In the case of survivin-ΔEx3, deletion of exon 3 results in a frame-shift with truncation of the BIR domain, as well as a modification of the COOH-terminal sequence, introducing a potential N-myristoylation site. As the BIR domain is proposed to be necessary for anti-apoptotic activity, it is unclear how survivin-ΔEx3 exerts its anti-apoptotic effect with a single truncated BIR domain, as shown when transfected into HepG2 cells [60]. A novel viral anti-apoptotic protein, K7 (also know as virus IAP *i.e.* vIAP), of Kaposi's sarcoma-associated herpesvirus has been proposed to be a survivin-ΔEx3 homolog, based on computational analysis [61]. The addition of the cryptic exon in survivin-2B also results in a modification of the BIR domain, introducing a potential N-glycosylation site, as well as 2 potential N-myristoylation sites. The resulting survivin-2B protein product, unlike survivin-ΔEx3, exhibits markedly reduced anti-apoptotic activity when expressed in HepG2 cells that were subsequently exposed to methotrexate. The reduced activity is possibly due to a survivin-2B dominant-negative mechanism of competitive (with survivin and survivin-ΔEx3) binding to the target site(s) [60, 62].

In studies of renal carcinoma cell lines, both survivin and survivin-ΔEx3 transcripts were detected in all cell lines tested, with survivin-2B mRNA present in most, but not all, cell lines included in the study [60]. Survivin, survivin-ΔEx3 and survivin-2B proteins were determined independently by Western blotting in both normal tissue (including smooth muscle, liver, breast and kidney) and neuroblastoma tissue (see "Expression of Survivin in human and murine tissues"). Equal levels of expression (mRNA and protein) of survivin-ΔEx3 and survivin-2B were reported in normal and tumor tissues [63]. However, as antibodies are not yet available that can be used to distinguish between the different splice variants at the protein level, by immunohistochemistry, most studies to date are based on analysis of the 3 survivin mRNA transcripts. In a study of 57 renal cell carcinomas (RCCs), including clear cell RCCs and less aggressive chromophilic/papillary RCCs, survivin, survivin-ΔEx3 and survivin-2B transcripts were reported to be present in all RCCs analysed, with survivin being the dominant transcript [64]. A stage-dependent relative decrease in survivin-2B mRNA was reported, although survivin mRNA levels could not be correlated with the grading and stage of clear cell RCCs. Similarly, a study of 30 gastric carcinomas was reported where survivin, survivin-ΔEx3, and survivin-2B mRNAs were detected in all tumors, with survivin being the dominant transcript [65]. While levels of survivin and survivin-ΔEx3 transcripts were unchanged with tumor stage, grade or histological type, a significant stage-dependent decrease in survivin-2B expression was evident. Based on analysis of human neuroblastoma cell lines and tumors, it was proposed that survivin-2B may act as a natural survivin antagonist, impairing or opposing the function of survivin in a dominant-negative manner [62].

The stage-dependent down-regulation of survivin-2B in human gastric [65] and renal [64] tumors and its lack of detection in soft tissue sarcomas [66] may be related to altered susceptibility to apoptosis induction in 2 ways: if survivin-2B acts as a natural anti-sense to survivin (as suggested by Islam *et al.* [62]), down-regulation of survivin-2B would result in a more significant anti-apoptotic effect by survivin; furthermore, as all survivin variants originate from a common hnRNA precursor, the decreased levels of survivin-2B may be associated with the generation of more anti-apoptotic survivin variants *i.e.* survivin and survivin-ΔEx3.

Further experimental research is necessary to elucidate the functional properties of the different splice variants. Results to date, however, suggest that alternative splice variants of survivin may be involved in the fine-tuning of survivin actions in human tumors.

Homologs of Survivin in Other Species

Homologs of survivin have been described in Drosophila (deterin), mouse (TIAP/m-survivin/ survivin$_{140}$), pig (survivin), and chicken (survivin-142). Alternative splice variants have also been described in some of these species, including survivin$_{121}$ and survivin$_{40}$ in mouse, survivin-Δ118bp in pig, and survivin-short, -δ and –γ in chicken.

Drosophila

In addition to the two Drosophila IAP-type proteins, DIAP-1 and DIAP-2, that contain the conventional IAP structure of multiple BIR and RING finger motifs [20, 40, 67], a survivin-type IAP *i.e.* containing a single BIR and no RING finger, termed deterin, has recently been described [68-69]. This 153 amino acid cytoplasmic protein (encoded by a 2 intron gene to an approximately 650 nucleotide message), apparently has widespread expression in early stage embryos, but becomes restricted to the gonads and central nervous system in later development. Deterin over-expression inhibits apoptosis induced in insect cells by either the natural caspase-dependent regulator *reaper* or by liposomal cytotoxants.

Mouse

Survivin expression has been detected in murine cells and tissues [46]. Due to its high level of expression in thymus and testis, murine survivin (m-survivin) has been designated TIAP [70]. M-survivin/TIAP is encoded by a gene containing 4 exons and 3 introns, located in the telomeric region of chromosome 11E2 that transcribes a 0.85

Kb mRNA, translating to a 16.2 kDa (140 amino acids) protein with 84 % homology with human survivin [70-71]. As with human survivin, the murine 5' flanking region of the survivin gene contains a TATA-less promoter containing a canonical CpG island [54, 72]. M-survivin/TIAP has one BIR and no RING finger. The α-helical-COOH 40 amino acid terminus predicts a coiled structure.

M-survivin/TIAP has been reported to be expressed in growing tissues such as thymus, testis and intestine of adult mice, and in many embryonic tissues [70]. Furthermore, m-survivin/TIAP expression, like human survivin, is upregulated at late S and G_2/M phases of the cell cycle ([70, 72]; see "Mechanism(s) of action of Survivin"). Thymocytes from transgenic mice overexpressing m-survivin/TIAP display enhanced proliferation in response to certain stimuli, including PMA and ionomycin, but not anti-CD3 antibody [73]. Using a low stringency genomic DNA hybridisation, Ogasawara *et al.* [74] identified a genomic DNA region on chromosome 9 that is 92% homologous to the mouse m-survivin/TIAP gene, and so was subsequently designated TIAP-2. Expression of TIAP-2 mRNA was detected in various murine tissues by RT-PCR; unlike that of m-survivin/TIAP, TIAP-2 mRNA levels were constant in the cell cycle, suggesting a different role for TIAP-2 from that of m-survivin/TIAP [74]. As for the human survivin variants, a complex regulatory balance has been proposed to exist between the different isoforms of m-survivin/TIAP and TIAP-2, determining the overall response to pro-apoptotic and proliferation stimuli [74].

Like in the case of the human survivin, three full-length murine survivin cDNA clones have been described, indicating the existence of three distinct survivin proteins [75]. The longest cDNA encodes a 140-amino acid residue protein ($survivin_{140}$), which is most highly homologous to human survivin in that it is derived from 4 exons and it contains a single IAP repeat and a COOH-terminal domain. Another cDNA retains intron 3, predicting a 121-amino acid residue protein ($survivin_{121}$) lacking the coiled-coil domain. The third cDNA encodes a 40-amino acid residue protein ($survivin_{40}$), lacks exon 2, and does not contain the IAP repeat and coiled-coil structure. Only $survivin_{140}$ and $survivin_{121}$ inhibit caspase-3 activity [75]. All three survivin mRNA splice variants were detected by RT-PCR analysis of mRNA derived from murine embryos at different developmental time points; $survivin_{140}$ being most prominent. RT-PCR analysis of a variety of adult murine tissues, including brain, heart, kidney, liver, lung, ovary, pancreas, spleen, testis, and thymus indicated high levels of $survivin_{140}$ mRNA in thymus and testis; $survivin_{121}$ mRNA was detected in all tissues analysed with strongest signals in brain and ovary. $Survivin_{40}$ mRNA, however, was not detectable in any of the adult murine tissues.

As described and consistent with previous reports for human survivin, expression of murine $survivin_{140}$ mRNA is most prominent in proliferating adult tissues. In contrast, murine $survivin_{121}$ mRNA transcripts appear to be more widely distributed, and have been found in most adult tissues analysed. Following immunoprecipitation of survivin from murine embryos (using an anti-survivin immunoglobulin that identifies all three recombinant forms), detection of bands representing $survivin_{140}$ and $survivin_{121}$ only were reported, confirming that in embryos two of the transcripts are translated into survivin proteins of the predicted sizes (approximately 16 kDa and 14 kDa) [75]. The immunoprecipitation, however, did not yield a band with a predicted molecular weight consistent with $survivin_{40}$.

Pig

Screening the expressed sequence tags database (dbEST) for sequences encoding previously unknown IAPs related to survivin, a putative porcine form of survivin *i.e.* the EST AJ 241166 clone from *Sus scrofa* small intestine cDNA containing an open reading frame for a protein with a single BIR motif has been described [76]. Using BLAST

Human MGAPTLPPAWQPFLK**DHRISTFKNWPFLEGCACTPERMAEAGFI**

Mouse MGAPALPQIWQLYLK**NYRIATFKNWPFLEDCACTPERMAEAGFI**

Pig MSAPSLPPAWQLYLK**DHRISTFKNWPFLEGCACTPERMAAAGFI**

Human **HCPTENEPDLAQCFFCFKELEGWEPDDDPIEEHKKHSSGCAF**LSVKKQFEELTLGEFL

Mouse **HCPTENEPDLAQCFFCFKELEGWEPDDNPIEEHRKHSPGCAF**LTVKKQMEELTVSEFL

Pig **HCPTENEPDLAQCFFCFKELEGWEPDDDPIEEHKKHSSGCAF**LSVKKQFEELTLSEFL

Human KLDRERAKNKIAKETNNKKKEFEETAKKVRRAIEQLAAMD

Mouse KLDRQRAKNKIAKETNNKQKEFEETAKTTRQSIEQLAA

Pig KLDKERAKNKIAKETNNKQKEFEETAKKVRCAIEQLAASE

Fig. (3). Protein sequence alignment of human survivin (Acc. No. AF077350), mouse survivin (Acc. No. AF077349), and pig survivin (Acc. No. AF195781) using CLUSTAL W program. Amino acids that differ in pig and mouse survivin from those in human survivin are underlined. The BIR domain is shown in **bold** [76-77].

searching, 91% homology between the porcine and human forms of survivin was predicted [77]. RT-PCR for the porcine sequence in intestine-derived cDNA confirmed the existence of the proposed mRNA, encoding a 142 amino acid protein. An additional faint band representing a smaller transcript was amplified. Direct sequencing of this product revealed a 118 nucleotide deletion. The deleted sequence showed high homology to the human survivin exon 3, indicating the presence of a survivin-ΔEx3 homolog in pig, termed survivin-Δ118bp. This suggests that there are equal modes of alternative splicing in humans and pigs [76]. CLUSTAL W comparisons among the pig, mouse and human protein sequences indicated that the sequences are highly conserved, particularly within the BIR domain (Fig. (3)). The expression of porcine survivin homologs in adult intestine tissue indicates that survivin and its splice variants may be expressed in non-neoplastic adult tissue at the RNA level.

Chicken

Similar to the discovery of porcine survivin, screening chicken (ch) dbEST derived from *Bursa of Fabricius* resulted in the identification of survivin homologs [78]. cDNAs for survivin-142 (homolog to human survivin), as well as 3 alternatively spliced variants *i.e.* survivin-short, -δ and –γ, have been described in chicken ovary cells. Survivin-142 mRNA expression is elevated specifically during the G2/M phase of mitosis and its transient overexpression protects hen granulose cells from taxol and C8-ceramide-induced caspase-3 activation and so apoptosis; overexpression of survivin-short (lacking much of the BIR domain and the entire α-helix coil domain) does not cause this effect. This suggests that survivin-142 is anti-apoptotic (see section on "Expression Of Survivin in Human and Murine Tissues"); while survivin-short has lost its anti-apoptotic potential.

The Subcellular Localisation of Survivin

Survivin is generally described as a cytoplasmic protein associated with poor prognosis in cancer, with fewer studies describing nuclear localisation and favorable prognosis. These findings may support the proposal that due to its differently folded structures (associated with its localisation), cytoplasmic survivin, but not nuclear survivin, is phosphorylated on Thr^{34}. This phosphorylation event is apparently necessary for apoptosis inhibition.

Although survivin has generally been described as a cytoplasmic protein [49, 79-85] and associated with unfavorable prognosis in cancer (see section on "Expression of Survivin in Human and Murine Tissues"), in agreement with a limited number of other research groups, we have found survivin to be predominantly located in the nucleus of cells, correlating with positive outcome in cancer [86-88]. Using a panel of antibodies raised against survivin epitopes including Cys^{57}-Trp^{67} (which is exposed and so reactive in cytoplasmic- and centrosome-associated survivin, but masked in nuclear and microtubule-bound survivin) and Ala^{3}-Ile^{19} (which is accessible in kinetochore-associated survivin, but not in cytoplasmic survivin), Fortugno *et al.* [89] reported that endogenously expressed survivin in Hela cells exists in distinct subcellular pools with approximately 80 % localised cytoplasmically and the remaining 20 % in the nucleus. It has been suggested that the differentially exposed epitopes may result from varying post-translational modifications of nuclear and cytoplasmic survivin *in vivo*. This is supported by the fact that only cytoplasmic survivin associates with (and is phosphorylated on Thr^{34} by) $p34^{cdc2}$-cyclin B1, a modification identified as a requirement for apoptosis inhibition [89-90]. This may correlate with our findings [88], and those of other research groups, where nuclear expression of survivin is an apparent indicator of a favorable outcome while survivin in the cytoplasmic region of cells may inhibit apoptosis and so correlate with worse outcome for the patient.

Although the panel of antibodies used in the study conducted by Fortugno *et al.* [89] can distinguish between nuclear and cytoplasmically-localised survivin, these antibodies cross-react with sequences present in survivin, survivin-ΔEx3 and survivin-2B. Our understanding of survivin and its endogenous role(s) may further be enhanced with the development of antibodies that independently recognise survivin and its splice variants.

Evidence for a Role of Survivin in Disease

Results from studies using cell line models support a role for survivin in cancer, where it may be associated with disease progression, angiogenesis and drug resistance. In multiple sclerosis (MS), survivin expression in T lymphocytes is associated with active disease states.

Survivin is a bifunctional protein capable both of regulating cell proliferation and suppressing apoptotic cell death. Several studies, involving survivin overexpression in cancerous and normal cells, *in vitro*, show inhibition of cell death induced by various apoptotic stimuli, including Fas (CD95), bax, caspases and anti-cancer drugs, with survivin down-regulation being associated with cell death (Table 1). Endogenous levels of survivin have been shown to be upregulated in response to such stimuli. For example, up-regulation of survivin mRNA was reported in *ex vivo* expanded $CD3^{+}$ $CD56^{+}$ T cells in response to Fas ligand interaction with the Fas receptor [91]; apoptosis resistance following lymphocyte activation correlates with survivin expression in memory T cells [92]; multidrug resistant HL60 cells (HL60R) overexpress survivin mRNA (but not p53 or bcl-2) compared to the wild-type HL60 cell line [93]; exposure of colorectal cell lines to X-irradiation, 5-fluorouracil and taxol results in up-regulation of survivin mRNA levels [94]; treatment of PC-3 prostate cancer cells with taxotere induced survivin protein expression within 4 hours of treatment [56]; and exposing the gastric cell line, MKN-45, to cisplatin resulted in increased expression of survivin mRNA and protein [95].

The mitogen, bFGF, induced an approximate 16-fold up-regulation of survivin mRNA and protein in human umbilical vein endothelial cells (HUVECs) [96]. Similarly,

Table 1. ***In vitro*** **Studies Indicating an Anti-Apoptotic Role for Survivin**

CELL LINE	TRANSECTION	APOPTOTIC STIMULUS	EFFECT	REFERENCE
293	Survivin cDNA (T)	Bax or Fas (co-transfection with survivin)	cell death partially inhibited (by 65+/-8%)	[139]
NIH3T3	Survivin cDNA (T)	taxol	↓ apoptosis	[26]
CHP134	Survivin cDNA (S)			[62]
HeLa, LnCAP NIH3T3, PC3 DU145	Survivin cDNA (T)	DR6-cDNA (TNF receptor) co-transfection	Inhibition of DR6-mediated apoptosis	[199]
HaCat	Survivin full-length anti-sense or dominant-negative (C84A) (T)	none	spontaneous apoptosis	[189]
YUSAC, LOX	Survivin full-length anti-sense or dominant-negative (C85A) (T)	none	spontaneous apoptosis	[175]
YUSAC-2	Survivin dominant-negative (T34A)-tet regulated (S)	spontaneous & cisplatin	↑ apoptosis & ↓ proliferation; inhibits tumor growth in SCID mice	[194]
HeLa	Survivin anti-sense (EPR-1 cDNA) (T)	spontaneous & serum-starvation	↑ apoptosis & ↓ proliferation	[52]
A549	Survivin anti-sense oligos (T)	etoposide	approx. 6-fold ↑ sensitivity to etoposide	[119]
MSN, TC620	Survivin anti-sense oligos (T)	spontaneous	↑ apoptosis by approx. 70-74%	[115]
JR8	Survivin ribozyme	cisplatin	↑ apoptosis	[120]
HeLa, HCT15 H460, HCT116 MCF-10, NHF HUVEC	Anti-sense oligos (T)	None	↑ apoptosis & polyploidy; ↓ colony forming in soft agar	[118]
BAEC	Survivin cDNA (T)	TNFα & cycloheximide	↓ apoptosis	[96]
HUVEC	Survivin cDNA; Survivin dominant-negative (C84A) (retroviral infection)	GF starvation; cisplatin; taxol	cDNA – protects v. apoptosis; C84A prevents protection	[200]
HUVEC	Survivin anti-sense oligos (T) with VEGF induction of survivin	C-6 ceramide; TNFα + cycloheximide	↑ apoptosis	[98]

Note: (T) = transient; (S) = stable; GF = growth factor; ↑ = increased; ↓ = decreased.

treatment of these cells with vascular endothelial growth factor (VEGF), a potent angiogenic factor, induced a 19-fold overexpression of survivin protein [97]. It has been proposed that the anti-apoptotic property of VEGF during angiogenesis in endothelial cells is primarily the result of induced survivin expression [98]. The significance of survivin in angiogenesis is further supported by studies on cultured microvascular endothelial cells (MVECs) [99] and HUVECs [100] indicating that the action of angiopoietin-1 (Ang-1), the endothelium-specific ligand essential for embryonic vascular stabilisation, branching morphogenesis, and post-natal angiogenesis, is associated with survivin up-regulation. Expression of a dominant negative form of survivin inhibits the ability of Ang-1 to protect cells from undergoing apoptosis. Overexpression of survivin (under the control of the keratin 14 promoter) in the epidermal keratinocytes of transgenic mice, resulted in resistance to ultraviolet B radiation indicating that survivin expression can inhibit cell death *in vivo* [101].

Progesterone treatment of breast cancer cells (T47-D) caused a down-regulation of survivin mRNA expression [102]. TNF-α induction of apoptosis in murine epidermal JB6-derived RT-101 cells involved the down-regulation of survivin expression at the protein level [103]. Induction of apoptosis in the ovarian cell line, SKOV3, by methylselenocysteine, was associated with survivin protein down-regulation [104]. The mechanism of action of genistein on the prostate cancer cell lines LNCaP and PC-3 has been shown, by cDNA microarray analysis, to involve the down-regulation of survivin [105]. Studies of peripheral T lymphocytes from patients with multiple sclerosis (MS) indicate that survivin levels are up-regulated in mitogen-stimulated T lymphocytes, and the levels correlate with MS disease progression. Interferon β -1a treatment, which down-regulates survivin protein expression, reduces clinical exacerbations of the disease [106-108].

These studies suggest a significant role for survivin in apoptosis and that the balance between surivivin's up-regulation/down-regulation may be critical in controlling cell viability and proliferation *versus* cell death.

Mechanism(s) of Action of Survivin

Cytoplasmic survivin phosphorylation at Thr^{34} is apparently necessary for its anti-apoptotic activity. The mechanism by which the anti-apoptotic activity is manifested is yet to be determined. Mechanisms involving direct or indirect suppression of caspase-3, -7 and -9 have been proposed, but have not yet been proven. The role of survivin in controlled cell proliferation apparently involves survivin translocation from the cytoplasm to the nucleus where its complex formation with cdk4 results in the release of p21 from its complex with cdk4. p21 subsequently inhibits caspase-3.

As survivin has been reported to be expressed at high levels in most common cancers and is described as the fourth most significant transcriptome expressed in human tumors [109], with low levels generally detectable in normal adult tissue, extensive research to elucidate the mechanism(s) of action of this potential drug target has been ensued. However, the mechanisms by which survivin regulates cell division and survival still remain controversial.

Survivin has been described as playing a role in the interface between cell cycle progression and apoptosis [90]. In proliferating cells, survivin is expressed in a cell cycle-regulated manner with high levels of expression in G2/M (40-fold up-regulation in this phase [28]) and rapid decrease in expression following cell cycle arrest. Survivin is proposed to counteract a default induction of apoptosis in G2/M phase. This process is apparently exploited in certain disease conditions including cancer, endometriosis, and MS, where the overexpression of survivin may overcome the apoptosis-related cell cycle check-point and favor aberrant progression of cells, which would otherwise be eliminated, through mitosis. At the beginning of mitosis, survivin apparently associates with the cyclin-dependent kinase $p34^{cdc2}$ and is phosphorylated on Thr^{34} by $p34^{cdc2}$-cyclin B1. It has been proposed that this phosphorylation of survivin on Thr^{34} is necessary to preserve cell viability and function [90]. Phosphorylated survivin subsequently associates with components of the mitotic apparatus, including centrosomes and the microtubules of the mitotic spindle [26]. In support of this, it has been shown (using *in vitro* mutational analysis) that a putative tubulin-binding domain exists in the extended survivin C-terminal α-helix [55]. Disruption of the interactions between survivin and the mitotic apparatus results in loss of anti-apoptotic function [110].

Survivin, endogenous [111] or transfected [112, 113], has also been identified at another sub-cellular location *i.e.* survivin has also been shown to associate with the midbody kinetochore of metaphase chromosomes and the central spindle midzone at anaphase, in a manner generally associated with chromosomal passenger proteins [26, 111-114]. Survivin apparently also binds cdk4 to aid in the G1/S transition, implicating survivin in normal G2/M and G1/S check-point transition and mitosis [26, 112, 115]. Support for these functional roles for survivin has come from *in vitro* cellular systems (see Table 1) where overexpression of survivin counteracts cell death induced by a range of stimuli, whereas disruption of survivin expression/function by anti-sense, ribozyme, or dominant negative mutants results in spontaneous apoptosis and multiple cell division defects with hyperploidy, supernumerary centrosomes, multipolar mitotic spindles, and multinucleation [116-120]. In studies of survivin's subcellular localisation, Fortugno *et al.* [89] described the majority (approximately 80 %) of endogenous survivin (in Hela cells) to be complexed with $p34^{cdc2}$-cyclin B1 and bound to microtubules of the mitotic spindle, with only 20 % being associated with kinetochores.

Controlling Apoptosis

The mechanism by which survivin inhibits apoptosis is not yet fully elucidated. Based on studies of recombinant survivin in cell-free systems, some reports suggest that survivin acts directly by suppressing caspases and pro-caspases – primarily caspase-3 and –7 [29, 75, 121] and by modulation of (and its modulation by) the transcription factor NF-kappa B [39, 122]. Other researchers have, however, suggested that based on similar analyses and the X-ray crystallographic structure, survivin does not appear to inhibit caspase-3 activity [55, 123, 124].

As the BIR domain of survivin is closely related to the 3D structure of the XIAP-BIR3 domain that binds and inhibits caspase-9 *in vitro* [57], it is possible that survivin functions, at least in part, by directly binding caspase-9. Supporting this proposed mechanism of action for survivin, is the demonstration in Hela cells that loss of phosphorylation of Thr^{34} by $p34^{cdc2}$-cyclin B1 results in dissociation of a survivin-caspase-9 complex on the mitotic apparatus and caspase-9-dependent apoptosis [90]. Furthermore, expression of the T34A dominant-negative mutant in cultured tumor cell lines leads to depletion of pro-caspase-9 [125]; conversely, survivin T34A dominant-negative mutant induction of apoptosis can be blocked by co-expressing a caspase-9 dominant-negative mutant [90]. However, in cell-free systems, the evidence that survivin can directly bind and inhibit purified caspase-9 is lacking. Furthermore, 3D structures of caspases fail to show a reason why Thr^{34} phosphorylation promotes interaction with caspase-9 [124].

An alternative explanation for the anti-apoptotic actions of survivin is that the effect on caspases is indirect. Survivin has been shown to bind Smac (Diablo), a mitochondrial pro-apoptotic dimeric protein known to bind IAPs and prevent their suppression of caspases [18, 126, 127], which could explain an indirect mechanism of survivin controlling caspases. The fact that overexpression of survivin T34A dominant-negative mutant induces mitochondrial cytochrome c release (an event upstream of caspase-9 activity), further indicates the complexity of survivin's mechanism of anti-apoptotic activity and the need for a better understanding of this process if survivin expression is to be exploited to its full potential as a target for therapy.

Regulating Cell Division

Although there is some evidence to suggest that survivin is involved in controlling cell cycle progression [117, 121,

128], the mechanism(s) by which survivin participates in this process are only beginning to be determined [129, 130]. It is apparent that, during the promotion of cell growth, survivin translocates from the cytoplasm into the nucleus, and there it interacts directly with the cell cycle regulator, cdk4, resulting in subsequent induction of Rb phosphorylation, cdk2/cyclin E complex kinase activation, and an accelerated S phase progression [131-132]. The survivin/cdk4 complex formation results in the release of the cyclin-dependent-kinase inhibitor, $p21^{Waf1/Cip1}$, from its complex with cdk4. p21 subsequently interacts with pro-caspase-3 to suppress apoptosis [133]. A number of studies support this proposed mechanism of action for survivin. Suppression of survivin/cdk4 complex formation results in the inhibition of cdk2/cyclin E complex kinase activation and up-regulation of the S phase population [121]. The use of a C84A dominant-negative survivin mutant or survivin anti-sense cDNA in studies of cultured cells produced results indicative of the disruption of a complex assembly of survivin, p21 and caspase-3, resulting in a caspase-dependent cleavage of p21 and so loss of normal mitotic progress [116]. It is clear, however, that further work is needed to understand and define survivin's role in apoptosis and cell division.

Expression of Survivin in Human and Murine Tissues

Survivin is expressed during fetal development and, at that stage, it is apparently essential for life. Studies of common human cancers have shown survivin protein expression in all cancer types studied, ranging from 35 % in gastric tumors to 100 % in malignant melanomas. Survivin protein is mainly described as cytoplasmic and an indicator of poor prognosis, whereas a few studies have reported nuclear localization and good outcome for patients. This, together with the fact that survivin has been detected in many normal cell types (to varying extents), must be considered when aiming to target survivin as a therapeutic approach to treat conditions such as cancer, endometrosis and MS.

Survivin has been shown to be abundantly expressed in proliferating fetal tissues during human and mouse development [46, 134, 135]. Studies of 14-21 week-old fetuses show survivin expression in epidermis, kidney proximal tubules, lung acinar tubules, pancreatic islets, intestinal crypts, thymic medulla, spinal cord neurons, axial mesenchymal space surrounding the somites and in the endometrial glands [46]. At E11.5, survivin was detected in murine brain and at E14.5, in lung and intestine, but it was no longer detectable by E15 [46, 70]. Transgenic murine embryos lacking survivin died before 4-5 days post-coitum, indicating that the presence of survivin is inevitable for life [136].

One of the most significant features described for survivin, to date, is its apparent differential distribution in cancerous as compared to normal tissues, with anti-survivin antibodies detected in sera from patients with lung, colorectal [137] and gastric [138] cancers. Analysis of approximately 60 cancer cell lines indicated survivin expression in all cell lines, with highest levels in those derived from breast and lung cancers [139]. In human cancers, as summarised in Table 2, survivin is overexpressed in tumors of the lung [140-142], breast [82, 88, 143], colon [79, 144-148], thyroid [149], stomach [80], esophagus [150], pancreas [85, 151], kidney [64, 152], liver [86], larynx [153], bladder [154, 155], uterus [156, 157], cervix [158], ovaries [159-161], prostate [162] and skin [84, 163], as well as leukemias [164-167], large B-cell lymphoma [83], soft-tissue sarcomas [66, 168], glioblastomas [169], and neuroblastomas [81, 170-174], compared to corresponding normal tissues. The percentage of survivin-positive patients varies greatly among the different tumor types studied – from approximately 35 % in a study of gastric tumors [80] to approximately 100 % in a study of malignant melanoma [175]. In the majority of studies, survivin protein has been described as cytoplasmic and associated with poorer prognosis, with a limited number of recent publications reporting mainly nuclear staining, indicative of favorable outcome in patients. The recent report that survivin (mRNA and protein) has an increased expression in a proportion of first-degree relatives of gastric cancer patients emphasises the importance of understanding the function role of survivin and its variants [176].

As indicated in Table 2, the level (mRNA or protein) and techniques used for analyses of survivin have varied significantly. Some studies to date have involved mRNA analysis by Northern blotting, RT-PCR or RNase protection assay; others analysed protein by immunohistochemistry, Western blotting or another antibody-based test; while some studies involve both mRNA and protein analyses. Furthermore, in some cases the analysis is of survivin mRNA only, while in a few cases, survivin-ΔEx3 and survivin-2B were also investigated. In our studies of survivin splice variant mRNA expression in breast tumor biopsies, we found survivin to be the predominant mRNA form, followed by survivin-ΔEx3, with a very few tumors expressing detectable levels of survivin-2B mRNA. The expression of these mRNAs, however, did not correlate statistically with patient outcome (our unpublished results). To date, there are no commercially available antibodies that allow independent studies of survivin, survivin-ΔEx3 and survivin-2B proteins. However, further studies of survivin and its variants at both the mRNA (preferably using microdissected tumor cells to avoid contamination from non-malignant cells that may bias results) and protein levels should help to confirm the prognostic and therapeutic implications of survivin in cancer.

In non-cancer tissue, expression of survivin is apparently modulated in a number of conditions and diseases. Using subtractive hybridization technologies, survivin mRNA is reportedly down-regulated in lymphocytes from normal tension glaucoma patients compared to those from healthy controls [177]. Survivin (mRNA and protein) is up-regulated in endometriosis and it is proposed that, in association with matrix metalloproteinases (MMPs), survivin may contribute to survival and invasion of endometriotic cells [178]. Survivin overexpression in activated T lymphocytes is apparently a feature of clinically active MS. Through a

Table 2. Summary of Survivin Expression in a Range of Human Cancers Types and Its Apparent Relevance to Patients Prognosis

TUMOUR TYPE	ANALYSIS	SURVIVIN EXPRESSION (%)	SUBCELLULAR LOCATION	RELEVANCE OF SURVIVIN EXPRESSION	REFERENCE
BLADDER	IHC	78	N/A	α recurrence & ↑ grade; no α p53 & bcl-2	[154]
BLADDER (in urine)	RT-PCR; antibody-based test	100 (15/15) 100 (31/31)	N/A	α ↑ grade	[155]
BREAST	IHC	70.7	cytoplasm	Poor survival; α with bcl-2	[82]
BREAST	IHC	60	nucleus -mainly	↑ relapse-free survival & overall survival	[88]
BREAST	RT-PCR	90.2 (23 in adjacent normal)	N/A		[143]
COLORECTAL	IHC	53.2	cytoplasm	poor survival rate; α bcl-2, not p53	[79]
COLORECTAL	RT-PCR	63.5 (29.1 in normal)	N/A	Poor survival rate	[145]
COLORECTAL	IHC	100 (adenoma &hyperplastic polys; & normal tissue)	cytoplasm - mainly	No α prognosis	[184]
COLORECTAL	IHC	2.3 adenoma- low dysplasia 52.4 adenoma-high dysplasia 63.3 carcinoma - adenoma	cytoplasm	↑ tumor progression	[148]
CNS	IHC	87.5	cytoplasm	No α tumor type, grade or patients prognosis	[201]
NEUROBLASTOMA	IHC	47	cytoplasm	↑ tumor stage & poor survival rate	[81]
NEUROBLASTOMA	Northern blot		N/A	α poor prognosis; ↑ tumor stage	[179]
NEUROBLASTOMA	RT-PCR		N/A	α poor prognosis	[173]
NEUROBLASTOMA	RNase protection assay	90 (that recurred) 27.7 (that didn't recur)	N/A	α recurrence	[174]
NEUROBLASTOMA	RNase protection assay	100 (7/7 that recurred) 0 (8/8 that didn't recur)	N/A	α recurrence	[171]
B-CELL LYMPHOMA	IHC	55	cytoplasm	N/A	[49]
B-CELL LYMPHOMA	IHC	60	cytoplasm	α poor prognosis	[83]
LEUKEMIA & LYMPHOMAS (ALL, CLL, AML, CML, NHL)	RT-PCR	76.2	N/A		[180]
LEUKEMIA	IHC	60	cytoplasm	α poor prognosis	[164]
LEUKEMIA	WB	88.8	N/A		[185]
SOFT TISSUE SARCOMA	RT-PCR	64 - Survivin 0 - Survivin-2B 42 - Survivin-ΔEx3	N/A	α poor survival rate	[66]
GASTRIC	IHC	34.5 (0 in adjacent normal)	cytoplasm	α bcl-2 & p53; ↓ apoptosis	[80]
GASTRIC	IHC	82 (nuclear) 88 (cytoplasm)	nuclear & cytoplasm	nuclear staining α good prognosis	[87]
GASTRIC	RT-PCR	13.3 (0 in normal)	N/A	No α apoptosis or survival	[202]
GASTRIC	RT-PCR	100 - Survivin 100 - Survivin-2B 100 - Survivin-ΔEx3	N/A	↓ survivin-2B levels α ↑ stage	[65]
SKIN	IHC	93	N/A	↑ aggressive tumor	[175]

(Table 2). contd.....

TUMOUR TYPE	ANALYSIS	SURVIVIN EXPRESSION (%)	SUBCELLULAR LOCATION	RELEVANCE OF SURVIVIN EXPRESSION	REFERENCE
SKIN	IHC	81 in BCC 92 in SCC			[189]
GASTRIC	IHC & WB	64 in skin SCC 56 in oral SCC	cytoplasm	↑ grade, size & spread to nodes	[84]
ESOPHAGEAL	RT-PCR	70.6 (47.1 in normal)	N/A	poor survival rate	[150]
LIVER	IHC RT-PCR	70 (14/20) 87.5 (7/8)	nuclear –mainly (weak cytoplasm)	α ↑ proliferation index	[86]
PANCREAS	IHC	76.9 in PDCA 56.3 in IPMT	cytoplasm	α ↓ apoptotic index	[85]
PANCREAS	IHC	88	cytoplasm (few cells – nuclear)	α ↑ proliferation index, ↓ apoptotic index & bcl-2; no α with p53	[151]
KIDNEY	RNase protection assay	50 (that recurred) 18 (that didn't recur)	N/A	α with recurrence	[152]
KIDNEY	RT-PCR	100 - Survivin 100 - Survivin-2B 100 - Survivin-ΔEx3	N/A	↓ survivin-2B levels α ↑ stage	[64]
OVARIAN	RT-PCR	86 (0 in 43 normal cases)	N/A		[159]
UTERINE & CERVICAL	RT-PCR	100 100	N/A N/A		[156]
UTERINE	IHC	43.9 – stage I; 49.3 – stage II; 73.4 – stage III; 88 – stage IV (normal: 0-5.1 proliferating; 0-15.8 secretory)	nuclear – mainly; some cytoplasmic	α ↑ stage, grade & poor survival rate	[157]

Note:
(1) This Table is not a comprehensive of all studies of survivin performed to date on clinical material;
(2) ALL = acute lymphocytic leukemia; AML = acute myelocytic leukemia; BCC = basal cell carcinoma; CLL = chronic lymphocytic leukemia; CML = chronic myelocytic leukemia; CNS = central nervous system; IPMT = intraductal papillary-mucinous tumor; PDC = pancreatic ductal cell adenocarcinoma; NHL = non-Hodgkin's lymphoma; RT-PCR = reverse transcriptase polymerase chain reaction; SCC = squamous cell carcinoma; WB = Western blot; α = correlates; ↑ = increased; ↓ = decreased; N/A = not applicable.

mechanism that apparently involves the down-regulation of survivin (but not bcl-2 or Fas), interferon β-1a treatment increases T cell susceptibility to apoptosis, reduces clinical exacerbations and also reduces the disease burden for the patient [106-108].

Survivin is often described as undetectable in terminally differentiated "resting" normal tissue [49, 115], but detection may depend on the sensitivity of the method used. Indeed, retinoic acid treatment of CHP134 neuroblastoma cells results in down-regulation of survivin mRNA levels, while survivin-2B levels remain constant [179]. Similarly, 5 days after retinoic acid plus TNF-induced differentiation of the malignant hematopoietic cell line, HL60, survivin mRNA levels were decreased to approximately 14 % of that in untreated cells [180]. Normal tissues shown to express survivin include thymus [49, 181], endometrium [182], cervix [183], basal colonic epithelium [184], endothelium [184], and $CD34^+$ cells derived from normal bone marrow [185, 186] and umbilical cord blood [187, 188]. There are conflicting reports as to whether or not survivin is expressed in basal keratinocytes [27, 101, 189]. Transcripts for survivin and its splice variant survivin-Δ118bp were detected in non-neoplastic adult (pig) intestine tissue [76]. Survivin mRNA was detected in noncancerous breast tissue (*albeit* at lower levels than in breast tumor tissue) [143], as well as normal colorectal [145], esophageal [150] and uterine [157] tissues (see Table 2). Fogt *et al.* [63] reported survivin, survivin-ΔEx3 and survivin-2B (mRNA and protein) to be equally expressed in both normal (lymph node, skeletal muscle, liver, breast, kidney) and tumor (neuroblastoma) cells.

The importance of studying not only survivin, but also its splice variants, at both the mRNA and the protein level remains to be determined. In general, the outcome of the documented studies support the significance of survivin (and possibly survivin-ΔEx3, but not survivin-2B) as a target for cancer therapy, which may further be expanded to other conditions including endometriosis and MS. The effects of such treatments on rapidly dividing normal cells, which may also express survivin, must be considered.

Regulating Survivin Expression and Potential Therapeutic Implications

A number of approaches aimed at regulating survivin expression as a potential therapy for cancer

have been studied in cell lines and animal models. These include anti-sense, ribozyme, and dominant-negative mutant technologies.

As described throughout this review, there are many mechanisms by which survivin may be controlled in the cell. These include epigenetic control through the canonical CpG region upstream of exon 1 [54]; EPR-1 and survivin-2B potentially acting as natural anti-sense [49, 52] and dominant negative [62] controls, respectively; inhibition of Thr^{34} phosphorylation by $p34^{cdc}$-cyclin B1, which apparently occurs only on cytoplasmic localised survivin [90]. A mechanism of survivin destruction *via* the ubiquitin-proteasome pathway has been described [190]. Wild-type p53, a nuclear transcription factor/tumor suppressor gene, has also been shown to repress survivin expression both at the mRNA and protein levels [191]. The mechanism(s) by which this action of p53 is achieved is not yet known [192, 193]. As p53 inhibits cdc2 expression during G2 arrest, which in turn is required for T^{34} phosphorylation, it may be that p53 also controls survivin at this level. Conversely, it has been proposed that loss of p53 [101] may result in increased phosphorylation of survivin and so inhibition of apoptosis [124].

Extensive research aimed at inhibiting survivin expression has been described, the objectives of which have been both to investigate the effects on blocking survivin expression and so increase our understanding of the complex mechanisms involved as well as for possible cancer therapeutic purposes. The documented studies performed in cultured cells include the use of anti-sense (oligos and full-length cDNAs), ribozymes and dominant-negative mutants, including targeting T^{34} phosphorylation (see Table 1). Expression of T34A dominant-negative in xenograft models of melanoma cancer in SCID mice suppressed *de novo* tumor formation, inhibited the growth of existing tumors by 60-70% by mechanisms involving inhibition of cell proliferation, and increased apoptosis *in vivo* [194]. As a potential cancer gene therapy tool, a replication-deficient adenovirus encoding the T34A dominant-negative (pAd-T34A) was generated by Mesri *et al.* [125]. Infection with pAd-T34A resulted in spontaneous caspase-9 and –3-mediated apoptosis in breast (MCF-7), lung (A549), cervical (Hela), colorectal (HCT116) and prostate (PC3) cancer cell lines, without affecting viability or proliferation of normal human cells, including fibroblasts, endothelium, or smooth muscle. pAd-T34A has been reported to be as effective as taxol and more effective than adriamycin in inducing tumor cell death. In *in vivo* xenograft models of breast cancer in SCID mice, pAd-T34A inhibited tumor formation and suppressed the growth of existing tumors by approximately 40 % [125]. Based on studies indicating spontaneous cytotoxic T-cell responses against survivin-derived Class I-restricted T-cell epitopes in breast, leukemia and melanoma patients, both *in situ* as well as *ex vivo*, it has been proposed that survivin may potentially be a suitable target for anticancer immunotherapy [195-198]. These results suggest that controlled repression of survivin, directly in tumors (see Tables 1 and 2), or by preventing angiogenesis (see section on "Evidence for a Functional Role of Survivin in Disease"), may be of significant benefit in cancer, and possibly other diseases, as a future therapy.

CONCLUSION

A lot is now known about survivin, its molecular organisation, homologs in non-humans, splice variants, expression (cell types and location) in normal and disease conditions, and mechanism(s) of action – controlling both proliferation and apoptotic cell death. Approaches to control its expression using anti-sense, dominant-negative mutants, ribozymes and immunotherapy have been investigated. Furthermore, the detection of survivin antibodies in sera from cancer patients suggests that such antibodies may have diagnostic potential.

A number of issues, however, remain to be resolved. Survivin is generally described as a potentially suitable target for anti-cancer therapy because of its high level of expression in many cancers (survivin expression is associated in some cases with favorable prognosis). This may also be true for other conditions/diseases, including MS and endometriosis. It is essential that before targeting survivin expression in patients, we understand the significance of the outcome of such action at all stages of the cell cycle, on all splice variants, and on normal tissues. Further research in this exciting field is necessary if the knowledge accumulated to date on survivin is to be exploited to its full potential in the future.

ABBREVIATIONS

ALL	=	Acute lymphocytic leukemia
AML	=	Acute myelocytic leukemia
Ang-1	=	Angiopoietin-1
BAEC	=	Bovine aortic endothelial cells
BCC	=	Basal cell carcinoma
BIR	=	Baculovirus IAP repeat
BIRPs	=	BIR-domain containing proteins
CARD	=	Caspase-recruiting domain
cDNA	=	Complementary DNA
ch	=	Chicken
c-IAP	=	Cellular IAP
CLL	=	Chronic lymphocytic leukemia
CML	=	Chronic myelocytic leukemia
CNS	=	Central nervous system
dbEST	=	Expressed sequence tags database
DIAP	=	Drosophila inhibitor of apoptosis
EPR-1	=	Effector cell protease receptor-1

EST	=	Expressed sequence tags
GF	=	Growth factor
HUVECs	=	Human umbilical vein endothelial cells
IAP	=	Inhibitor of apoptosis
IPMT	=	Intraductal papillary-mucinous tumor
MMPs	=	Matrix metalloproteases
mRNA	=	Messenger RNA
MS	=	Multiple sclerosis
m-survivin	=	Murine survivin
MVECs	=	Microvascular endothelial cells
N/A	=	Not applicable
NIAP	=	Neuronal apoptosis inhibitor
NHL	=	Non-Hodgkin's lymphoma
pAd-T34A	=	Replication-deficient adenovirus encoding T34A dominant-negative mutant
PDC	=	Pancreatic ductal cell adenocarcinoma
PKA	=	Protein kinase A
PKC	=	Protein kinase C
RCC	=	Renal cell carcinoma
RT-PCR	=	Reverse transcriptase-polymerase chain reaction
S	=	Stable
SCC	=	Squamous cell carcinoma
SCID	=	Severe combined immunodeficient mice
T	=	Transient
TNF	=	Tumor necrosis factor
UCD	=	Ubiquitin-conjugating domain
VEGF	=	Vascular endothelial growth factor
WB	=	Western blot
X-IAP	=	X chromosome-linked inhibitor of apoptosis

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