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Clinico-Pathologic Relevance of Survivin Splice Variant Expression in Cancer

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Abstract

Survivin is a member of the inhibitor of apoptosis (IAP) family and has multifunctional properties that include aspects of proliferation, invasion and cell survival control. Survivin is a promising candidate for targeted cancer therapy as its expression is associated with poor clinical outcome, more aggressive clinico-pathologic features, and resistance to radiation and chemotherapy. In the present review the different properties of the Survivin splice variants are discussed and their activities correlated with different aspects of cancer cell biology, to include subcellular location. Special emphasis is placed on our current understanding of these Survivin splice variants influence on each other and on the phenotypic responses to therapy that they may control.

Expression of Survivin in Cancer

For the past 15 years, Survivin expression in cancer has been the subject of intense research due to its widely recognized anti-apoptotic properties and attractive potential as a therapeutic target. Survivin expression in diverse tumor tissues has been linked with shorter overall and disease-free survival[1; 2; 3; 4; 5]. Survivin can contribute to tumor progression by greatly enhancing the chemo- and radiation resistance characteristics of solid tumors including breast[6], laryngeal squamous cell carcinoma (LSCC)[7], and colorectal cancers[8; 9]. Our research has demonstrated that acquired apoptotic resistance to a multitargeted tyrosine kinase inhibitor as well as other structurally unrelated FLT-3 inhibitors paralleled Stat3 mediated Survivin overexpression in acute myeloid leukemia. [10]. Recently, we also reported that cervical carcinoma cells can secrete Survivin, generating an encapsulated extracellular pool of Survivin which can be taken up by other cancer cells enhancing their anti-apoptotic, proliferative, and metastatic potential[11; 12].

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Conflict of interest Statement

None

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Currently, three therapeutic strategies targeting survivin in cancer through immunotherapy, small molecule inhibitors, or antisense oligonucleotides are being evaluated or have completed Phase II clinical trials[13].

Survivin Splice Variants

The discovery of Survivin splice variants with apparently distinct functional roles has sparked a great interest in ascertaining the roles of these Survivin isoforms in solid tumors as well as hematological malignancies. The fact that homologs of Survivin splice variants appear to be conserved among other species[14], suggests a fundamental importance of Survivin splice variants functional roles. At least 6 alternative splice variants of the survivin gene have been reported in humans (Figure 1). The mature wild-type (WT) survivin transcript composed of 4 exons which are spliced together to form a 426 bp coding transcript that is translated into a protein of 142 amino acids[15]. The exon/intron arrangements of the other survivin splice variants differ in the following ways. Survivin 2B is generated by introducing a 69 bp nucleotide fragment from intron 2, creating an additional and alternative exon named 2B[16]. Survivin 3B is generated by introducing a 165 bp nucleotide fragment from intron 3, creating an alternative exon named 3B[17]. Survivin DeltaEx3 is generated by alternative splicing which skips over 102bp of exon 3 and leads to a frameshift in the translation in the carboxyl end of the protein[16; 18]. Survivin 2 is generated by introducing a 197 bp tail from intron 2 of which only two of the nucleotides are coding since this contains an early stop codon[15]. Likewise, Survivin 3 is generated by introducing a 209 bp terminus from intron 2[2; 19].

Survivin Splice Variant Sequence Homology

Strikingly, all of the Survivin protein isoforms arising from the splice variants share complete sequence identity in the N-terminus region, including some or all of the Baculovirus IAP Repeat (BIR) domain, yet they differ in the carboxyl end (Figure 1). Analysis of similarities and differences between WT survivin and the other variants may provide further information about the functional capabilities and limitations of these variants. As shown in Figure 1, all Survivin splice variants conserve specific residues within the BIR domain that have been linked to important antiapoptotic roles in WT Survivin. This includes S20 which is a target for cytosolic cyclic AMP-dependent protein kinase A (PKA) phosphorylation[20]. S20 phosphorylation disrupts Survivin and XIAP binding which affects antiapoptotic activities[20]. Another important residue shared by all Survivin splice variants is T34, which is phosphorylated by p34(cdc2)-cyclin B1[21]. Mutations which impede phosphorylation of Survivin at this position, initiate a cascade of cellular changes that induce the mitochondrial apoptotic pathway[22]. Phosphorylation of T48 by the protein kinase CK2 is necessary for the anti-apoptotic functions of WT Survivin in HeLa cells[23]. Both L64[24] and D71[25], which are also conserved among all Survivin splice variants, are critically involved in binding the apoptotic effector Smac/Diablo[26].

Only two of the Survivin splice variants, 2B and 3B, share sequence identity in the carboxyl domain (after P73) with WT Survivin (Figure 1). This part of the sequence includes at least two important antiapoptotic domains. First, K79–G83 identified as an Hsp90 binding region[27]. Disruption of the Survivin-Hsp90 complex initiates mitochondrial apoptosis[28]. A second region, V89–L98, contains a Crm-1 dependent NES sequence[29]. Intriguingly, deacetylation of residue K129 in WT Survivin favors the formation of Survivin monomers which can then heterodimerize with Crm-1 for active nuclear export[30]. Conversely, acetylation of K129 promotes homodimerization and nuclear accumulation of WT Survivin[30]. Furthermore, the K129 acetylated Survivin homodimer binds the STAT3 dimer and represses transactivation of STAT3 target gene promoters[30]. Currently, it is

unknown if any of the Survivin splice variants have the ability to homodimerize, but as discussed below, splice variant expression is frequently associated with antiapoptotic functions in cancer.

Clinico-pathologic Characteristics of Survivin Splice Variant Expression

It has been shown that both chemotherapy and radiation regimens can have an effect on the expression pattern of survivin splice variants in cancer cells[18] [31]. Specific expression patterns of survivin splice variants observed prior to treatment, as well as after treatment have been significantly linked to several clinic-pathologic characteristics. For example, in breast cancer it has been observed that patient response to two different chemotherapies is significantly associated with distinct splice variant expression patterns[18]. Furthermore, significant correlations to disease prognosis found in the splice variant expression patterns can change after one course of chemotherapy[18]. In uterine cervical carcinoma tumors, an inverse correlation between irradiation dose and levels of WT Survivin, and differences in splice variant expression patterns are detected among patient samples[31]. The transcript expression levels of various survivin variants have been significantly associated with clinico-pathologic characteristics in several cancers (Tables 1–4). Given their early discovery[16], the majority of studies have focused on analyzing expression levels of survivin splice variants deltaEx3 (Table 1) and 2B (Table 2).

Survivin deltaEx3

The prognostic significance of deltaEx3 expression has been investigated in at least 13 types of cancer (Table 1). With the exception of one study which linked a higher deltaEx3/survivin ratio to improved sensitivity to Docetaxel + Epirubicin treatment in breast cancer[18], all studies associate deltaEx3 expression levels with adverse clinical factors and/or unfavorable prognosis (Table 1). In comparison to normal tissues, higher levels of deltaEx3 expression have been detected in many malignant tissues such as breast[5], colorectal[3; 32], cervical[33], and thyroid[34] cancers. Expression levels have been linked to advanced pathological grades and clinical stages of bladder cell carcinoma[35], prostate cancer[36], glioblastoma[36], and breast cancer[5]. A potential role for deltaEx3 in tumor invasiveness and metastasis of bladder[37] and colorectal[38] cancers has also been observed. deltaEx3 is linked to shorter progression-free and overall survival outcomes in some cancers including childhood AML[4] and soft tissue sarcomas[39].

Survivin 2B

Reports describing the clinico-pathologic relevance of survivin 2B expression in cancer are conflicting (Table 2). While some authors report a stage dependent upregulation of 2B expression in colorectal carcinoma[3] as well as a grade dependent 2B upregulation in diffusely infiltrating astrocytoma[2], associated with adverse survival outcomes in both instances, others have linked high levels of 2B expression with positive clinical outcomes in the same cancers[40; 41]. Specifically, increased levels of 2B expression have been associated with benign, rather than malignant brain tumors[40] and a significant correlation to a better prognosis in colorectal carcinoma[41]. In fact, about half of the studies investigating expression of 2B in cancer, report an inverse correlation between expression levels of this splice variant and adverse clinical outcomes (Table 2). Expression levels of survivin 2B mRNA have been negatively correlated with the pathologic grades and clinical stages of bladder cell carcinoma[35], lymph node involvement in breast cancer[5], and higher risk in pediatric ALL[42]. In contrast, a positive correlation between survivin 2B expression and adverse survival outcomes is observed in adult AML[4], and soft tissue sarcomas[39].

Survivin 2 α

Expression of survivin 2 α has been linked to adverse outcomes in several cancer types (Table 3). In colorectal cancer 2 α expression levels are reported to increase significantly in advanced tumor stages[3; 32]. In breast cancer, 2 α expression is associated with poor relapse-free survival[5], and resistance to chemotherapy[18]. When compared to normal controls, survivin 2 α transcripts have been found to be anywhere from 2 to 95 times more abundant in cancer cell lines derived from breast, osteosarcoma, lung, acute lymphocytic leukemia, colorectal, and medulloblastoma cancers, and up to 150 times greater in medulloblastoma tissue samples[15].

Survivin 3B

Expression of survivin 3B has been detected in at least three types of cancer (Table 4). When compared with normal mucosa, a slightly increased level of survivin 3B expression was detected in oral squamous cell carcinoma, however the difference did not reach levels of statistical significance[43]. In colorectal carcinoma tumors, the expression levels of survivin 3B were significantly higher than normal tissue, however these authors did not detect any other associations to the clinico-pathologic features[3; 32]. In breast cancer, increased levels of 3B expression are associated with a shorter overall and disease-free survival[5; 18; 44].

Survivin 3 α

Very little is known about the expression of survivin 3 α in cancers. Expression of this splice variant was initially detected in the NB4 acute promyelocytic leukemia cell line, but no other observations were reported[19]. Analysis of 73 brain neoplasms containing diffusely infiltrating astrocytoma failed to detect expression of the 3 α variant[2]. Most recently, an intriguing study found expression of the survivin 3 α to be specifically detected in breast tumor samples, while none was found in matching adjacent normal breast tissues[45], suggesting a potential role for this variant in breast cancer development.

Do Survivin splice variants have the ability to heterodimerization with WT Survivin?

Survivin dimerization occurs spontaneously between WT Survivin monomers in solution[46], however the functional significance of the Survivin homodimer *in vivo* has not been well defined[47]. The structural relevance of the Survivin dimer in cell cycle progression as well as apoptosis has been investigated. Surprisingly, it has been shown that it is the monomer form of WT Survivin that is critical to several cellular processes, notably efficient nuclear export[48], and efficient binding of the mitochondrial apoptotic protein, Smac/Diablo[49]. In contrast, Survivin homodimerization seems to be important for other cellular functions such as microtubulin stability[49], resistance to chemotherapy compounds that interfere with tubulin formation[15], and interaction with STAT3 in the nucleus[30]. Recently, it has been suggested that a controlled balance between the monomer and dimer forms of WT Survivin regulates the divergent functions of this protein in different cell types[49].

Co-immunoprecipitation and pull-down assays have revealed that at least two of the Survivin splice variants, DeltaEx3 and 2B, have the ability to bind to WT Survivin[50; 51; 52]. Another study shows that a third variant, 2 β , may also heterodimerize with WT Survivin and alter its antiapoptotic function[15]. It is widely hypothesized that Survivin splice variant heterodimerization could displace one of the homodimer protein partners and disrupt normal function[51], however the biological significance of a physical interaction

between WT Survivin and any variant is still unknown. WT Survivin homodimerization occurs when L98 from one of the Survivin monomers protrudes into a hydrophobic pocket formed by L6, W10, F93, F101, and L102 of the other Survivin molecule, and mutations affecting the hydrophobic pocket block homodimerization[49]. The majority of the residues required to form this hydrophobic pocket are not conserved in the DeltaEx3, 3 or 2 sequences (Figure 1) so WT heterodimerization with these splice variants would certainly involve the interaction of distinct residues. At least one group has reported that this interaction involves the BIR domain of DeltaEx3[52]. It has not been reported which protein regions may be involved in the WT and 2B interactions observed in HeLa cells expressing these variants[50; 51]. However, the presence of an additional 23 amino acids within the hydrophobic pocket region of Survivin 2B (Figure 1) makes it unlikely that this structure is conserved in this variant or involved in WT heterodimerization. Until crystal structure resolutions of these protein interactions become available it is not possible to determine which residues are involved in heterodimerization with WT Survivin.

Subcellular Localization of WT Survivin

The subcellular location of Survivin has been shown to have an important influence on tumor biology. Survivin has been found in the mitochondria of cancer cells[53; 54], but not of normal tissues suggesting that this subcellular compartmentalization has a role in tumor pathogenesis[6]. In response to apoptotic stimuli, mitochondrial Survivin is rapidly released into the cytosol where it prevents caspase activation and inhibits apoptosis[53]. Although initially believed to bind directly to the caspases through its BIR domain[55], Survivin has not been shown to act such under physiological conditions[25; 53]. Instead, Survivin uses its BIR domain to bind to and stabilize another anti-apoptotic protein, XIAP, which then interacts with and inactivates the apoptotic caspases[56]. Nuclear and cytoplasmic localization of Survivin may also influence tumor progression. Nuclear compartmentalization of Survivin is frequently associated with a poor clinical prognosis[57; 58; 59; 60; 61; 62], however, directly conflicting results[63; 64] suggest this issue requires further investigation.

Subcellular Localization of Survivin Splice Variants

It has been difficult to study the subcellular localization of the Survivin splice variants, to better understand their functions and evaluate their effects on tumor biology, because few antibodies that detect specific Survivin isoforms have been developed[2]. In addition available Survivin splice variant commercial antibodies are polyclonal and react with several other nuclear and cytoplasmic proteins making it difficult to quantify expression and location of Survivin variants[65]. Only a few studies have reported the use of DeltaEx3 or 2B antibodies to confirm the presence of these Survivin variants in protein extracts obtained from distinct subcellular fractions of Daoy[50] or HeLa[66; 67] cancer cell lines, as well as paraffin embedded tumor tissues[68]. However, the majority of Survivin splice variant subcellular analysis has been done with genetic constructs that control exogenous expression and allow for Western blotting or immunofluorescent detection of recombinant protein products.

Forced exogenous expression of WT survivin, 2B, and deltaEx3 in Ishikawa cells, showed that WT Survivin and 2B are primarily located in the cytoplasm while DeltaEx3 has a distinct nuclear accumulation[31]. The same pattern was shown in HepG2 hepatoma cells expressing GFP-tagged Survivin variants[69]. Furthermore, these authors reported that a bipartite nuclear localization signal (NLS) found exclusively in the DeltaEx3 sequence may be involved in cytoplasmic clearance of this variant[69]. In one analysis of Daoy medulloblastoma cells and HeLa cervical cancer cells, GFP-DeltaEx3 was observed to

localize exclusively to the nucleus; while GFP-2B localized to both nuclear and cytoplasmic compartments in Daoy cells, and exclusively to the cytoplasm in HeLa cells[50]. Others have reported DeltaEx3 localization to both the nucleus and mitochondria[67]), or to all subcellular compartments (nucleus, cytoplasm and mitochondria) in HeLa cells expressing this survivin splice variant[52]. It has been shown that WT Survivin, 2B, and 3B contain a Crm-1 dependent nuclear export signal, which DeltaEx3 and 2 are lacking [70]. GFP-Survivin, GFP-2B and GFP-3B are predominantly cytoplasmic and accumulate in the nucleus of HeLa cells if Crm-1 activity is inhibited, while the location of DeltaEx3 (predominantly nuclear) and 2 (nuclear/cytoplasmic) is unaffected[70].

The results of these and other studies have revealed intriguing insights into potential molecular mechanisms influencing the apoptotic functions of Survivin splice variants in cancer, particularly in the case of DeltaEx3, where multiple methods by which this splice variant may contribute to tumorigenesis have been reported. Mitochondrial accumulation of DeltaEx3 in cancer cells is believed to function in inhibition of mitochondrial-dependent apoptosis[50]. In fact, the structure of the DeltaEx3 protein contains a mitochondrial localization signal and a unique BH2 domain required for binding to the apoptotic effector Bcl-2 [50]. In HeLa cells exposed to the DNA damaging apoptotic agent etoposide, an increase in mitochondrial and nuclear accumulation of DeltaEx3 was shown[67]. Here, DeltaEx3 was found to have a role in delaying the release of the pro-apoptotic molecule Smac/Diablo from the mitochondria, while knockdown of DeltaEx3 expression sensitized HeLa cells to etoposide induced apoptosis[67]. The delayed release of Smac/Diablo did not appear to be regulated by Bcl-2 since mitochondrial levels of this protein decreased with etoposide exposure. Another group also reported that DeltaEx3 was able to prevent release of cytochrome C and Smac/Diablo in LIGHT/IFN- γ treated Hep3B2T cells, showing that DeltaEx3 and Smac/Diablo could be co-precipitated in mitochondrial fractions of these cells[71]. In HeLa Cells expressing HA-tagged DeltaEx3, it was shown that this isoform could be detected in all subcellular fractions and protected cells from TNF- α -induced mitochondrial apoptosis, by acting through a caspase-3 dependent pathway[52]. Furthermore, it was shown that a deleted DeltaEx3 recombinant protein containing only its interrupted BIR domain was sufficient to pull down recombinant active caspase-3, while full length recombinant DeltaEx3 (but not a BH2 deleted mutant) could be immunoprecipitated with endogenous Bcl-2 [52]. These researchers found that endogenous DeltaEx3 could act as an adaptor, using its BH2 and BIR domains to link Bcl-2 to active caspase-3, protecting cells from the caspase-3 apoptotic pathway.[52]. In various tumor cells transfected with FLAG-tagged DeltaEx3, this splice variant was isoform was reported to localize exclusively to the nuclei, and be phosphorylated at multiple sites by the checkpoint kinase Chk2 during etoposide induced DNA damage[36]. Site directed mutagenesis of the residues thought to be phosphorylated by Chk2 was found to enhance DeltaEx3 stability, inhibit expression of phosphorylated H2AX which senses and recruits repair proteins to areas DNA damage, and impair cell growth after exposure to the etoposide stimuli[36]. Another research group also reported that degradation of DeltaEx3 was linked to its anti-apoptotic functions in cancer cells, finding that the DeltaEx3 sequence contains a nucleolus localization signal, and localized to these areas of the nucleus when cells were treated with proteasome inhibitors [72]. They found that DeltaEx3 was degraded by the ubiquitin–proteasome pathway, which was necessary for its anti-apoptotic role in resistance to doxorubicin[72]. In non-cancer cells DeltaEx3 has also been reported to have an important regulatory role in processes affecting vascular endothelial cell spreading, migration, and angiogenic capabilities[68] which may also contribute to tumorigenesis. In this study, DeltaEx3 was only detected within the vascular endothelial cells surrounding tumor cells, during an analysis of paraffin embedded pediatric brain tumor tissues[68]. Subsequent assays revealed that this splice variant had a role in vascular tube formation in vitro, and angiogenesis in vivo, although it did not appear to influence apoptosis or proliferation of endothelial cells[68]. In all, these studies provide

substantial evidence of multiple cellular functions and mechanisms by which expression of DeltaEx3 may be involved in tumorigenic processes.

Forced exogenous expression of survivin splice variants influences cell viability

While none of the Survivin splice variants has the ability to rescue cell proliferation inhibited by antisense RNA-mediated WT Survivin depletion[51], in some cases, forced exogenous expression has affected cell viability and provided evidence about the pro- or anti-apoptotic functions of Survivin splice variants. In the A431 epidermoid carcinoma cell line, forced expression of 3B was shown to have a cytoprotective effect in cells exposed to cisplatin or irradiation[70]. These results suggest that the 3B variant has an anti-apoptotic function in cancer cells and may play an important role in their survival[70]. In contrast, an apoptotic function has been associated with 2B expression[54]. Forced expression of the 2B variant was shown to interfere with tubulin polymerization and induce mitochondria-dependent apoptosis in HeLa cells[54]. In addition, overexpression of 2B was shown to sensitize cells to taxol-induced cell growth inhibition and cell death, while siRNA silencing of endogenous 2B transcripts made cells resistant to taxol treatment[54]. Similarly, overexpression of 2B in the EU-4 (acute lymphocytic leukemia) cell line inhibited cell growth and sensitized cells to doxorubicin-induced apoptosis[73]. Transfection of Daoy cells with a 2 expression vector interfered with the anti-apoptotic functions of WT Survivin in co-transfection assays, even without exposure to a cell death stimulus, vincristine[15]. In HeLa cells overexpressing deltaEx3, a dual and antagonistic function was observed; while DeltaEx3 was shown to decrease etoposide induced apoptosis, it also interfered with tumor spheroid formation by decreasing cell proliferation and clonogenic survival[74].

Regulation of survivin differential splicing

The molecular mechanisms involved in alternative splicing of the survivin transcript are not well characterized. Presumably, the factors that drive differential splicing of the survivin gene come into play during transcription as multiple transcription initiation sites creating mRNA variants have not been identified for survivin, as they have for other important oncogenes, such as the epigenetic regulator BORIS[75]. The human genome contains a 15 kb copy of the survivin gene located on chromosome 17 q25[47; 76] and all splice variants appear to be derived from the same initial transcript. Several regulatory elements of the survivin promoter have been identified including numerous Sp1 binding sites and cell-cycle dependent regulatory regions[77]; however, only p53 expression has been reported to alter expression patterns survivin splice variants[73]. In addition, p53 mutation status is significantly associated with increased expression of specific survivin splice variants in breast cancer[78]. Typically, p53 mutations are associated with high levels of survivin expression[79], while wild-type p53 suppresses survivin expression[80]. A direct physical interaction between wild-type p53 and DNTM1 has been shown to influence DNA methylation levels of the survivin promoter and represses survivin expression in HCT116 cells[81]. This is important because the DNA methylation patterns of the survivin gene may have a key role in the regulation of alternative splicing of survivin transcripts.

The influence of DNA methylation on the splicing process and spliceosome activities was recently described[82]. The spliceosome is a ribonucleoproteic complex consisting of more than 100 proteins and at least 5 small nuclear ribonucleic acids (snRNAs) that splice the nascent mRNA transcript in a complex dynamic process that has not been well characterized[83]. The splicing process takes place in the nucleus at the same time as transcription and has recently been linked to an elaborate intragenic DNA methylation pattern that promotes RNA polymerase II transcriptional “pausing” observed to favor exon

inclusion[82]. This transcriptional delay likely allows recruitment of certain spliceosome components that influence selection of exon/intron sites[82]. For survivin, both methylation patterns of the CpG rich promoter[84; 85; 86], and intragenic methylation surrounding exon 1 of the survivin gene[79; 87; 88] have been described. It is possible that p53 binding to the survivin promoter may be involved in the recruitment of specific spliceosome components to this region. At least one study has shown that p53 physically interacts with the spliceosome protein SAP145 which can affect pre-mRNA splicing sites[89]. Further investigation is necessary to discern the extent to which DNA methylation or p53 may influence the survivin splicing process.

Discussion/Conclusions

Understanding the role of Survivin splice variants in cancer and their clinico-pathological relevance has been a topic of great interest and substantial investigation. Analyses have been complicated by lack of reliable tools (antibodies) to evaluate quantities and subcellular localization of Survivin isoforms effectively. Forced expression of Survivin splice variants in cancer cells reveals distinct subcellular compartmentalization patterns[70] that shift upon exposure to death stimuli[67]. Co-expression of Survivin variants can also influence subcellular compartmentalization patterns[15; 50]. This shift is thought to influence the anti-apoptotic functions of the splice variants, although their specific cellular activities are not yet understood. Furthermore, it is not known whether the splice variants exist as monomers or homodimers or which physiological conditions favor heterodimerization with WT Survivin. Most analyses of survivin splice variant expression in tumor samples reveal significant associations with adverse clinico-pathologic characteristics in diverse cancers (Tables 1–4). However, conflicting study outcomes, particularly in regards to survivin 2B expression (Table 2), highlight the need to perform additional analyses at the protein level. As more tools become available to facilitate the study of these intriguing proteins, we will undoubtedly gain a better understanding of their cellular activities, role in tumorigenesis, and their influence on clinical prognosis. It is unclear if targeting one specific variant over another will prove useful given that survivin splice variants all originate with the same promoter. As long as the strategies completed in the Phase II trials target WT Survivin, expression patterns of variants are expected not to influence the outcomes. This knowledge has the potential to influence current therapeutic strategies targeting expression of survivin in cancer. If future analyses at the protein level reveals that expression of a particular splice variant, it may be strongly associated with favorable clinical outcomes.

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Figure 1.

Multiple alignment of Survivin splice variants. Many residues and regions important to the anti-apoptotic functions of WT Survivin are highlighted and shared with other splice variants (*indicates the residue is common to all isoforms). This includes much of the BIR domain highlighted in the WT sequence (F13–V89), the XIAP binding region (K15–M38), and S20 which can disrupt Survivin and XIAP binding when phosphorylated[20]. Other functionally important residues include T34 which can also be phosphorylated[22], T48[23], Smac/Diablo binding residues L64[24] and D71[25], as well as the K79–G83 Hsp90 binding region[27], and V89–L98 Crm-1 dependent Nuclear Export Signal (NES) sequence[29].

Table 1

Clinico-pathologic characteristics associated with deltaEx3 expression levels.

Cancer	Comparison to Normal Tissues/Cells	Pathological Grades and Clinical Stages	Adverse Survival Outcomes	Resistance to Chemotherapy	Metastasis or Tumor Invasiveness
Breast Cancer		(275) [5] (35) [45] Malignancy		(60) [18] Tax-Epi	
Colorectal Carcinoma	(47) [3; 32]	(47) [3; 32] (52) [38]			
Diffusely Infiltrating Astrocytoma	(73) [2]		(73) [2]		
Oral Squamous Cell Carcinoma	(22) [43]				
Non Small Cell Lung Cancer		(134) [90]			
Childhood AML			(31) [4]		
CML		(51) [91]			
Thyroid Carcinoma	(61) [34]				
Bladder Cell Carcinoma		(60) [35]			(30) [37]
Glioblastoma		(49) [36]			
Prostate Cancer		(150) [36]	(150) [36]		(150) [36]
Cervical Carcinoma	(20) [33]				
Soft Tissue Sarcoma			(76) [39]		
Brain tumor	(25) [40]				(25) [40]

The number of tumor samples analyzed in each study is shown in parenthesis. Arrows indicate direction of correlation (=Positive; =Negative). Numbers in brackets display the reference from which the information was obtained.

Table 2

Clinico-pathologic characteristics associated with survivin 2B expression levels.

Cancer (# Samples)	Comparison to Normal Tissues/ Cells	Pathological Grades and Clinical Stages	Adverse Survival Outcomes	Resistance to Chemotherapy	Metastasis/Tumor Invasiveness
Breast Cancer	(35) [45]			(60) [18] Tax-Epi	(275) [5]
Colorectal Carcinoma	(47) [3; 32]	(47) [3; 32] (51) [92] (52) [41]			
Diffusely Infiltrating Astrocytoma	(73) [2]		(73) [2]		
Ovarian Cancer				(6 cell lines) [93] Taxane	
Prostate Cancer				(PC3 cell line) [65] Doxorubicin & Radiation	
Oral Squamous cell carcinoma	(22) [43]				
Pediatric B-ALL	(20) [42].				
Adult AML			(51) [4]		
CML		(51) [91]			
Brain Tumor		(25) [40] Malignancy			
Renal Cell Carcinoma		(57) [69]			
Gastric Carcinoma	(76) [94]	(30) [95] (30) [96] (76) [94]			
Bladder Cell Carcinoma		(60) [35]			
Cervical Carcinoma	(20) [33]	(20) [33]			(20) [33]
Soft Tissue Sarcoma			(76) [39]		

The number of tumor samples analyzed in each study is shown in parenthesis. Arrows indicate direction of correlation (=Positive; =Negative). Numbers in brackets display the reference from which the information was obtained.

Table 3

Clinico-pathologic characteristic associated with survivin 2 expression levels.

Cancer	Comparison to Normal Tissues/Cells	Pathological Grades and Clinical Stages	Poor Survival Outcomes	Resistance to Chemotherapy	Metastasis/Tumor Invasiveness
Breast Cancer	(35) [45]		(275) [5]	(60) [18] FEC	
Medulloblastoma	(7) [15]				
Colorectal Carcinoma	(47) [3; 32]				
Diffusely Infiltrating Astrocytoma	(73) [2]		(73) [2]		

The number of tumor samples analyzed in each study is shown in parenthesis. Arrows indicate direction of correlation (=Positive; =Negative). Numbers in brackets display the reference from which the information was obtained.

Table 4

Clinico-pathologic characteristics associated with survivin 3B expression levels.

Cancer	Comparison to Normal Tissues/Cells	Pathological Grades and Clinical Stages	Poor Survival Outcomes	Resistance to Chemotherapy	Metastasis/Tumor Invasiveness
Breast Cancer	(35) [45]		(275) [5] (60) [18] (135) [44]		
Colorectal Carcinoma	(47) [3; 32]				
Oral Squamous Cell Carcinoma	(22) [43]				

The number of tumor samples analyzed in each study is shown in parenthesis. Arrows indicate direction of correlation (=Positive; =Negative). Numbers in brackets display the reference from which the information was obtained.