

Survivin – biology and potential as a therapeutic target in oncology

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Abstract: Survivin is a member of the inhibitor-of-apoptosis proteins (IAPs) family; its overexpression has been widely demonstrated to occur in various types of cancer. Overexpression of survivin also correlates with tumor progression and induces anticancer drug resistance. Interestingly, recent studies reveal that survivin exhibits multiple pro-mitotic and anti-apoptotic functions; the differential functions of survivin seem to be caused by differential subcellular localization, phosphorylation, and acetylation of this molecule. In this review, the complex expression regulations and post-translational modifications of survivin are discussed. This review also discusses how recent discoveries improve our understanding of survivin biology and also create opportunities for developing differential-functioned survivin-targeted therapy. Databases such as PubMed, Scopus® (Elsevier, New York, NY, USA), and SciFinder® (CAS, Columbus, OH, USA) were used to search for literature in the preparation of this review.

Keywords: survivin, BIRC5, IAP, XIAP, caspase-9, Samc, DIABLO

Introduction

Survivin (encoded by baculoviral inhibitor of apoptosis repeat-containing 5 [*BIRC5*]) is a member of the inhibitor-of-apoptosis proteins (IAPs; Figure 1) family found ubiquitously distributed during embryonic and fetal developmental stages. Many studies have shown that survivin, unlike other IAPs, is prominently expressed in the vast majority of neoplasms but not in the differentiated normal tissue. Its overexpression has been demonstrated in breast and lung cancer, prostate, gastric, colon, bladder and esophageal carcinomas, osteosarcomas, and lymphomas.¹⁻⁴ Overexpression of survivin was also found to be significantly associated with a poor prognosis and decreased survival rates in oral, breast and colorectal cancers.^{5,6} In fact, it has been revealed in past studies that patients with survivin overexpressed had a more aggressive and metastatic colorectal carcinoma.^{6,7} In addition, Kawasaki et al⁷ demonstrated that survivin plays an important role in the transition of colorectal tumorigenesis. Another study done by Hsu et al⁸ demonstrated that in oral cancer, the extent of survivin expression was negatively correlated with the degree of differentiation. Therefore, survivin overexpression seems to play important roles in the pathogenesis and the progression of various types of cancer.

Because the expression of survivin is positively correlated with tumor progression and inversely correlated with the survival period of the patient after chemotherapeutic treatments, it is important to determine both the molecular regulations and functions of survivin. The survivin gene encodes a 16.5 kDa protein consisting of an N-terminal Zn²⁺-binding baculovirus inhibitor of apoptosis protein repeat (BIR) domain linked to a

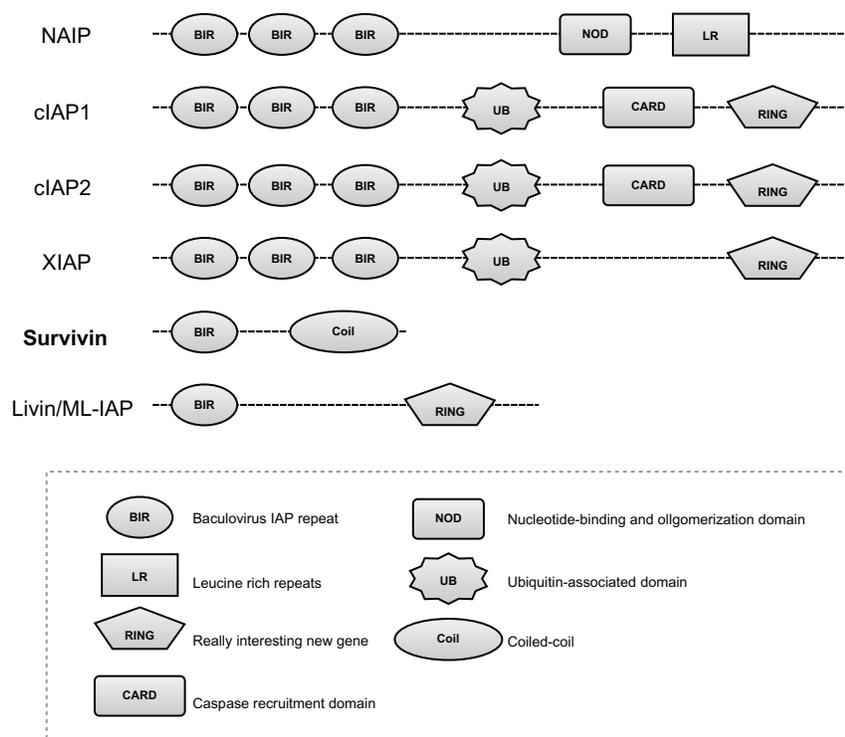


Figure 1 IAP family members.

Note: Proteins of the inhibitor-of-apoptosis (IAP) family include NAIP, c-IAP1, c-IAP2, XIAP, survivin, and ML-IAP/Livin.

65 Å amphipathic C-terminal α -helix.⁹ Originally, survivin was thought to inhibit caspase-dependent apoptosis through cooperation with the X-linked inhibitor of apoptosis protein (XIAP) and interference with caspase-3/-9. Survivin can also promote mitosis by forming the chromosomal passenger complex (CPC) with aurora-B kinase, inner centromere protein (INCENP), and Borealin. However, recent studies reveal that survivin exhibits multiple anti-apoptotic and pro-mitotic functions, and that the differential functions of survivin seem to be caused by differential subcellular localization, phosphorylation, and acetylation of this molecule. The basic functions of survivin and the current development (both preclinical and clinical) of various survivin inhibitors such as YM155 and SPC3042 for cancer therapy have been discussed in our previously published review.¹⁰ In this review, we will mainly discuss the complex expression regulations and post-translational modifications of survivin. We will also discuss how recent discoveries have improved our understanding of survivin biology and also create opportunities for developing differential-functioned, survivin-targeted therapy.

Transcriptional and translational regulation of survivin expression

Survivin can be regulated at various levels, including transcriptional regulation, post-translational modification, and

protein stability regulation. It has been widely demonstrated that deregulation of survivin gene expression is associated with tumorigenesis. Therefore, it is crucial to understand the underlying molecular mechanisms of survivin gene expression and regulation.

Previous studies indicated that regulation of the survivin gene occurs at the level of transcription.¹¹ Specificity protein 1 (Sp1) is a human transcription factor involved in gene expression in various cellular development processes. It binds directly onto the DNA promoter region and enhances gene transcription. Given that the survivin promoter lacks a typical TATA or CCAAT box and that Sp1 plays a role in the regulation of genes lacking a functional TATA box,¹² it is thought that the survivin gene contains several putative Sp1/Sp3 transcription factor-binding sites. In fact, Xu et al¹³ have revealed that there are two essential binding sites for Sp1 on the survivin promoter region (one at position -148 to -153; the other at position -127 to -140) in human HeLa cervix adenocarcinoma cells, which suggested that Sp1 is essential in regulating basal survivin promoter activity. In fact, a previous study using electrophoretic gel mobility shift assay (EMSA) showed that Sp3, a closely related homologue of Sp1, regulates the survivin promoter in concert with Sp1.¹³ The same study also revealed that increasing Sp1 or Sp3 levels would induce endogenous survivin expression.

In contrast, inhibiting Sp1 or Sp3 levels by mithramycin or ribonucleic acid interference (RNAi) would decrease the survivin promoter activity.¹³ Interestingly, another study done by Chen et al¹⁴ demonstrated that overexpression of Sp1 in A549, a human lung adenocarcinoma cell line, increased survivin expression at both messenger (m)RNA and protein level. This study also revealed that silencing Sp1 by mithramycin or RNAi decreased survivin expression. Taken together, the Sp1 family serves as an important factor in mediating survivin promoter activity.

The survivin promoter region harbors binding sites for a range of regulatory proteins. Apart from Sp1 regulation, evidence of p53 and retinoblastoma (RB)/E2F pathway being involved in survivin gene regulation has also emerged. Using rat embryonic fibroblasts, scientists demonstrated that overexpression and binding of E2F activators (E2F1, E2F2, and E2F3) to the E2F-like binding element increased survivin promoter activity. In contrast, mutation in the E2F DNA-binding domain abolished E2F-induced survivin promoter activity.¹⁵ Considering the repressive property of RB on E2F activators, Jiang et al¹⁵ also demonstrated that RB-inactivation by E1A abrogated the inhibitory effect on survivin transcription in rat embryonic fibroblasts cells. Moreover, the same study revealed that both E2F activators (E2F1 and E2F3) and E2F repressor proteins (E2F4 and E2F5) were able to bind onto the promoter of survivin in human WI-38 embryonic fibroblasts,¹⁵ suggesting that survivin transcription can be positively regulated by E2F activator proteins and negatively regulated by E2F repressor proteins. Another study had also identified an E2F-like binding element spanning from -63 to -53 upstream of the human survivin transcription start site, and both p53 and RB were shown to be essential in repressing survivin transcription in normal human melanocytes.¹⁶ Because the p53-binding element, located on the -96 to -5 region of survivin promoter, overlaps with an E2F-like binding element, it has been suggested that p53 interferes with the binding between E2F activators and survivin promoter, and subsequently suppresses E2F-mediated survivin gene transcription. Interestingly, recent works on survivin reveal that activation of γ -secretase induces proteolytic cleavage of Notch, resulting in the release of the Notch-intracellular domain (NICD) and the subsequent translocation of NICD to the nucleus to activate survivin genes transcription.^{17,18}

Evidence also points to non-transcriptional mechanisms controlling survivin levels in tumor cells. Vaira et al¹⁹ demonstrated that insulin-like growth factor-1 (IGF-1) up-regulated survivin expression by promoting translation of survivin in the human DU145 prostate cancer cell line. Since previous

studies had shown that IGF-1 promoted cellular proliferation through activating phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling pathway,²⁰ Vaira et al treated prostate cancer cells with rapamycin, an mTOR inhibitor, to investigate whether the mTOR pathway was involved in the regulation of IGF-1-induced survivin expression. Their data showed a novel survival circuitry initiated by IGF-1/AKT signaling, and involving mTOR activation, p70S6K activation and increased translation of the survivin mRNA.

Regulations of survivin protein stability

Cell-cycle dependent regulation of survivin

In addition to regulating survivin expression at the transcriptional and translational levels, its expression can also be regulated through altering protein stability. Survivin is shown to be clearly regulated by the cell cycle, as its expression is found to be dominant in the G2/M phase.²¹ Interestingly, some studies show that the up-regulation of survivin in the G2/M phase is mainly caused by reduced rate of protein degradation instead of increased rate of gene transcription in cells.²² It was demonstrated in a study done by Zhao et al²² that the level of endogenous survivin was increased in human embryonal kidney 293 cells arrested in the G2/M phase. In contrast, the level of endogenous survivin was shown to be greatly reduced in 293 cells arrested in the G1 phase. Furthermore, the reduction in survivin levels during G1 phase was abolished by the application of proteasome inhibitors, MG132 and lactacystin.²² On the basis of these findings, cell cycle dependent pattern of survivin expression and degradation was proposed to be associated with the ubiquitin-proteasome pathway.

Heat shock protein 90 mediated survivin protein stability

Cells express several molecular chaperones to ensure the correct folding of proteins upon protein synthesis. The chaperones are also required to refold proteins that can become denatured when cells are exposed to stresses such as abnormally high temperatures. Heat shock protein 70 (Hsp70) and heat shock protein 90 (Hsp90) are the most important molecular chaperones in mammalian cells. Unlike Hsp70, Hsp90 does not serve to fold nascent proteins. Rather, Hsp90 binds to substrate proteins that are in a near-native state and thus at a late stage of folding.²³ Some scientists have suggested that

the adenosine triphosphate (ATP)-bound state of Hsp90 binds stably to substrate polypeptides that are held by an internally dimerized clamp, and substrate release is achieved through the process of ATP hydrolysis and associated conformational changes within Hsp90.²³ Survivin associates with Hsp90 *in vitro* via an interaction that involves the ATPase domain of Hsp90. Evidence of direct binding between Hsp90 and survivin is supported by an enzyme-linked immunosorbent assay (ELISA)-based receptor-ligand assay.²⁴ The expression of both Hsp90 and survivin in HeLa cells increased in a time-dependent manner after heat shocking the cells. In contrast, treatment of HeLa cells with an Hsp90 inhibitor geldanamycin resulted in a concentration-dependent loss of survivin expression. Importantly, the loss of survivin after Hsp90 inhibition was also shown to be caused by proteasomal-dependent destruction.²⁴

Growth factor-induced survivin protein stability

So far, we have discussed the effect of Sp1/Sp3, RB/E2F, mTOR, and Hsp90 on the regulation of survivin expression. However, that is not the end of the story. Recent studies reveal that the proteasomal degradation of survivin can also be affected by activation of various growth factor signaling pathways. For example, a study carried out by Wang et al²⁵ showed that treatment with nutrients and growth factors, including glucose and epidermal growth factor (EGF), prompted an increased survivin protein expression in both mouse MIN6 and rat INS-1 pancreatic β -cells without significant increase in survivin mRNA levels or activation of survivin promoter. Moreover, the addition of EGF was shown to prevent polyubiquitination of survivin, thereby protecting survivin from degradation.²⁵ Studies done by Ju et al²⁶ and Siddiq et al²⁷ also consistently unmasked the relationship between the stability of survivin and EGF receptor (EGFR) signaling pathway in human breast cancer cells. Both studies revealed that survivin expression was up-regulated via extracellular signal-regulated kinases (ERK) activation and PI3K signaling in EGFR2 (HER2) activated human breast cancer cells through altering the protein stability of survivin. As we have discussed in previous sections, in response to cell death stimulation, survivin physically associates with XIAP; this complex in turn promotes enhanced XIAP stability and synergistic inhibition of caspase-9 activation. In contrast, the XIAP-associated factor 1 (XAF1) is a putative tumor suppressor that antagonizes the anti-apoptotic molecule XIAP. XAF1 binds to XIAP and subsequently activates XIAP's

ubiquitin-protein isopeptide ligase (E3) activity to catalyze survivin ubiquitination. Activation of HER2/ERK was shown to reduce the formation of the XIAP-XAF1 complex, which catalyses ubiquitin-dependent degradation of survivin, leading to an increase in survivin protein stability.²⁶ The regulation of survivin protein stability by HER2 is even more complicated: HER2 not only activates the ERK signaling pathway, but it also activates PI3K/AKT pathway.²⁶ The HER2/AKT pathway prompts a phosphorylation cascade that increased cyclin-dependent kinase 1 (CDK1/p34^{cdc2})-cyclin B1 phosphorylation on threonine 161 (Thr161) and subsequently increased the phosphorylation of survivin on Thr34.²⁶ As reported in previous studies (and as will be discussed in the following sections), phosphorylation on Thr34 is required to maintain the stability of the protein.²⁸ Thus, HER2/ERK and HER2/AKT signalling pathways act in concert to enhance the stability of survivin. Some scientists have also suggested that in addition to EGF, survivin expression can also be regulated by the vascular endothelial growth factor (VEGF) and the platelet-derived growth factor (PDGF).²⁹⁻³¹ The results of a study by Beierle et al²⁹ showed a consistent finding where exposure to VEGF induced PI3K/AKT pathway activation, which resulted in an increased survivin protein expression in neuroblastoma cells.

Post-translational modifications of survivin

Phosphorylation-diversified activities of survivin

It has been widely demonstrated that survivin is overexpressed in nearly all kinds of cancer cells, with minimal expression in the differentiated normal tissues. In addition, survivin plays important roles in both the process of mitosis and the inhibition of apoptosis. Although it has long been known that mutation in the promoter region of survivin and deregulation of its related transcription factors may lead to the overexpression of survivin in cancer cells,³²⁻³⁴ the mechanisms underlying the transition of survivin functions were largely unknown in the past. Recent works on survivin reveal that post-translational modification including phosphorylation and acetylation plays an important role in regulating cellular function of survivin. In fact, survivin can be phosphorylated at multiple sites (eg, threonine 34, 53, and 117; serine 20) and that these modifications can drive survivin to exhibit different molecular functions.

Phosphorylation of survivin on Thr34 by CDK1/p34^{cdc2}-cyclin B1 was observed in the human cervix adenocarci-

noma cells, oral submucous fibrosis and oral squamous cell carcinoma cells.³⁵⁻³⁷ Unlike other members of the IAPs, survivin only contains a single IAP-specific BIR domain, and the amino acid residue Thr34 lies within this domain. O'Connor et al³⁵ showed that Thr34 phosphorylated survivin bound to caspase-9 in HeLa cells. In contrast, Thr34 phosphorylation defective survivin was shown to be incapable of forming a complex with caspase-9. Furthermore, a phosphorylated dead mutation Thr34→alanine (T34A) survivin was developed in the same study, where the researchers demonstrated that the T34A survivin mutant could induce caspase 9-dependent apoptosis in HeLa cells.³⁵ Therefore, it is suggested that survivin phosphorylation on Thr34 by p34^{cdc2} may regulate intrinsic apoptosis (Figure 2). In fact, a recent study also shows that phosphorylation of survivin at Thr34 enhances its cytoprotective effect in cancer cells.³⁸

On the other hand, phosphorylation of survivin on serine 20 (Ser20) and threonine 117 (Thr117) displays distinct functions comparable to the phosphorylation mentioned previously. Two studies have indicated that protein kinase A (PKA) and polo-like kinase 1 (PLK1) catalyze the phosphorylation of survivin on Ser20.^{39,40} PKA is a serine/threonine kinase; its activity is associated with increased cell survival. Scientists have shown that PKA phosphorylates survivin on Ser20 in the cytosol, but not in mitochondria. In mammalian cells, survivin is located in both the mitochondria and the cytosol,

and mitochondrial survivin seems to play an important role in inhibiting the process of apoptosis.⁴¹ It is believed that in response to pro-apoptotic stimulation, mitochondrial Ser20-dephosphorylated survivin is rapidly released into the cytosol, where it prevents caspase activation and inhibits apoptosis through physical interactions with XIAP. A study done by Dohi et al³⁹ demonstrated that PKA-mediated Ser20 phosphorylation interfered with the binding capacity between survivin and XIAP in the cytosol of INS-1 cells. In addition, transfection of non-PKA phosphorylatable survivin was shown to be capable of enhancing XIAP stability and subsequently inhibit apoptosis.³⁹ Therefore, phosphorylation of survivin at Ser20 decreases its anti-apoptotic activity.

Interestingly, Chu et al⁴⁰ have illustrated that survivin Ser20 is also one of the substrates of PLK1. PLK1 is a multitasking protein that can regulate mitotic entry, centrosome separation, spindle assembly, and chromosome alignment. Besides PLK1, the chromosomal passenger complex (CPC) is also a key regulator of mitosis; this complex is composed of survivin, Borealin, INCENP, and Aurora B kinase.^{42,43} In the study carried out by Chu et al,⁴⁰ PLK1-mediated phosphorylation of survivin was shown to catalyze Aurora B activation. Subsequently, Aurora B attached to spindle microtubule and formed a CPC, which plays an important role in cytokinesis.^{40,44} Taken together, phosphorylation of survivin on Ser20 seems to regulate the differential functions

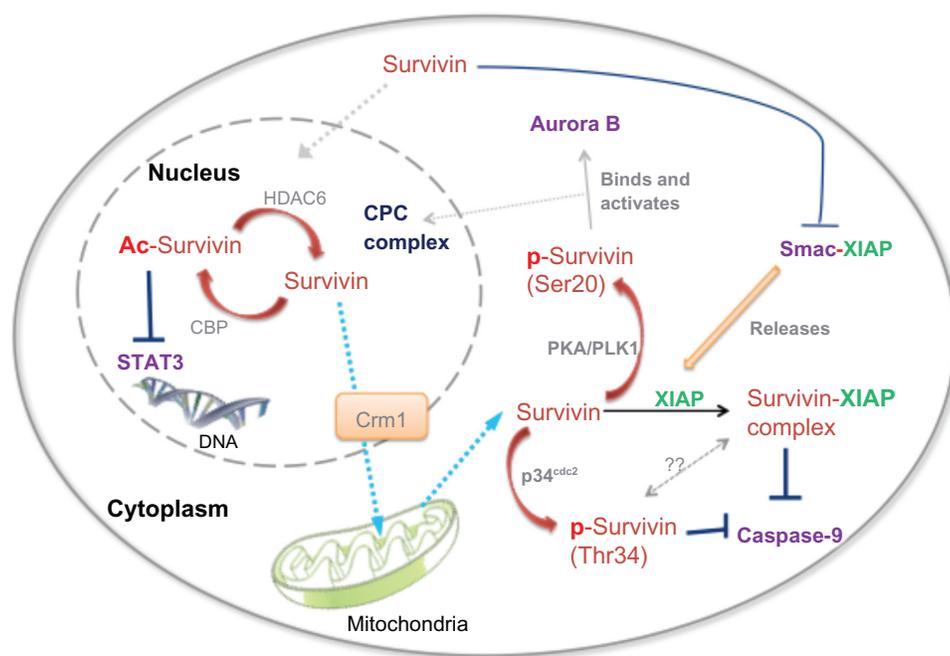


Figure 2 Post-translational regulations and functions of survivin.

Abbreviations: CPC, chromosomal passenger complex; STAT3, signal transducer and activator of transcription 3; Crm1, chromosome region maintenance 1; PKA, protein kinase A; PLK1, polo-like kinase 1; Smac, second mitochondria-derived activator of caspases; XIAP, X-linked inhibitor of apoptosis protein; HDAC6, histone deacetylase 6; Thr, threonine; Ser, serine; CBP, CREB-binding protein; Ac-Survivin, acetylated-Survivin; p-Survivin, phosphorylated-Survivin.

of survivin in both apoptosis and mitosis (Figure 2). It is also worth noting that Aurora B could in return phosphorylate survivin on Thr117 in CPC.^{45,46} Delacour-Larose et al⁴⁶ have demonstrated that the survivin phosphorylation dead mutant, T117A, could associate with centromeres and induce prometaphasic arrest in a survivin-depleted cell.

Acetylation of survivin

As we have discussed in the previous sections, survivin is present in both the mitochondria and the cytosol. In fact, survivin can also shuttle between the nucleus and the cytoplasm through active nucleocytoplasmic transport. Chromosome region maintenance 1 (Crm1/Xpo1) is a nuclear export receptor. It can interact with proteins containing the leucine-rich nuclear export signals and facilitates their nuclear export.⁴⁷ Crm1 is also a critical Ras-related nuclear protein (Ran-GTP) effector for mitotic spindle assembly and function in cells.⁴⁸ Early studies revealed that survivin contains a nuclear export signal (possibly sequences: ⁸⁹VKKQFEELTL⁹⁸ or ⁹⁶LTLGEFLKL¹⁰⁴) located between the BIR domain and the COOH-terminal α -helical coil, and it is exported to the cytoplasm through an interaction with Crm1.^{49,50} In addition, abrogation of the cytoplasmic translocation of survivin has been shown to promote caspase-dependent apoptosis in cancer cells. It was also shown that: (1) survivin dimerization occurs when leucine (Leu)98 of one of the molecules protrudes into a hydrophobic pocket, formed with Leu6, tryptophan (Trp)10, phenylalanine (Phe)93, Phe101, and Leu102 of the other molecule; and (2) homodimerization of survivin decreases its binding affinity to Crm1 and inhibits the nuclear export of survivin.^{42,51} However, factors affecting the transition between monomer and dimer of survivin were unclear. Interestingly, recent studies reveal that cAMP response element-binding protein (CREB)-binding protein (CBP) acetylates survivin on lysine 129 position to promote survivin homodimerization and the subsequent nuclear accumulation (Figure 2). These studies also reveal that cytoplasmic survivin inhibits apoptosis, whereas nuclear survivin may inhibit cell survival.⁵² It is not surprising to see that survivin could inhibit apoptosis in the cytoplasm because caspases such as caspase-9 and caspase-3 are mainly present in the cytoplasm. However, it is surprising to find that nuclear survivin could inhibit the transcription of various pro-survival genes in cancer cells. The Wang et al⁵² study reveals that CBP promotes survivin acetylation in the nucleus. In addition, the acetylated form of survivin binds to the N-terminal of signal transducer and activator of transcription 3 (STAT3) and represses its oncogenic activity. In contrast, deacetylation of survivin inhibits its homodimerization and promotes heterodimerization of survivin with Crm1 with

subsequent nuclear export.⁵² The same research group also found that histone deacetylase 6 (HDAC6) plays an important role in survivin deacetylation and the subsequent nuclear export of survivin (Figure 2). CBP-dependent nuclear translocation of HDAC6 was shown in MCF7 breast cancer cells following estrogen treatment to regulate the acetylation state of survivin in the nucleus.⁵³ It is widely accepted that nuclear survivin has reduced stability as compared with the cytoplasmic survivin,^{54,55} and that survivin promotes mitosis through formation of the CPC complex (together with INCENP and aurora B kinase) in the nucleus. In contrast, cytoplasmic survivin inhibits apoptosis through direct/indirect inhibition of caspases.^{54,55} Therefore, it will be important to determine whether the amount of survivin present in the nucleus is enough to induce a pro-apoptotic effect through STAT3 inhibition in cells in the future.

Possible mitosis- and apoptosis-unrelated functions of survivin

Growing evidence indicates that survivin interferes with the process of cell autophagy and down-regulation of survivin may induce apoptosis through autophagy-dependent mechanisms.^{56,57} An interaction between the autophagy regulator beclin-1 and survivin has been shown in human glioma cells in response to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL).⁵⁸ Interestingly, a recent study shows that XIAP inhibits autophagy via the E3 ubiquitin-protein ligase (Mdm2)-p53 signaling pathway. Thus, survivin may also indirectly inhibit autophagy through interactions with XIAP and interference with the Mdm2-p53 signaling pathway.⁵⁹ Besides mitosis, apoptosis and autophagy, a recent report shows that the translocation of survivin into the nucleus may enhance DNA double strand breaks (DSB) repair capability in cancer cells by up-regulating the molecular sensor of DNA damage, Ku70.^{60,61} However, the mechanism/s in which survivin regulates autophagy and DNA repair remains to be determined.

The potential of survivin as a therapeutic/drug target

“Druggable” proteins do not necessarily become therapeutic targets, and vice versa, promising therapeutic targets do not necessarily become druggable proteins. Although survivin is an important oncogenic molecule widely expressed in various types of tumor and its overexpression is positively correlated with the progression and metastasis of tumor in patients; survivin was only considered as a “semi-druggable” target in the past because: (1) survivin does not contain a kinase domain (ie, not a protein kinase); and (2) survivin exhibits multiple functions. Therefore, it is difficult to use any high-throughput

assay (eg, *in vitro* kinase assay) or molecular assay (eg, Western blot analysis) to screen potential survivin inhibitors.

However, improved understanding of the biology of survivin does create opportunities for scientists to develop different survivin-targeted therapies with differential cellular or molecular functions. For example, delivering phosphorylation-dead mutation survivin, T34A, into cancer cells may be a potential cancer therapeutic strategy. In theory, the T34A survivin mutant should be capable of forming a heterodimer with the wild-type survivin, and subsequently reducing the interaction between survivin and caspase-9. In fact, several studies have revealed the anti-cancer effect of delivering T34A plasmid DNA or recombinant protein by different nanocarrier approaches. Zhang et al⁶² successfully transported mouse-survivin-T34A plasmid DNA by utilizing heparin-polyethyleneimine (HPEI) nanoparticles. Survivin-T34A-loaded HPEI was shown to be capable of reducing colon cancer growth *in vitro* and *in vivo*.⁶² In addition, T34A mutant survivin could also enhance the chemotherapeutic sensitivity of drugs, including cisplatin and doxorubicin, in lung cancer cells and breast cancer cells.^{63,64} Besides the T34A mutant, a dominant-negative survivin (Cys84→Ala, C84A) protein fused to the cell-penetrating poly-arginine (R9) peptide has also been synthesized and was shown to be capable of inducing apoptosis in cancer cells.⁶⁵ Interestingly, scientists have demonstrated that transduction of double point mutant (T34A and C84A) of survivin by adenovirus induced anti-cancer growth effect in hepatocellular cancer cells.⁶⁶ In fact, survivin T34A/C84A mutant (with double mutations) was shown to exhibit a stronger cancer killing effect as compared with the T34A or C84A survivin mutant

(single mutation). Survivin contains a single BIR domain, and it is believed that the BIR domain plays an important role in physically interacting between survivin and second mitochondria-derived activator of caspases (Smac)/DIABLO.⁶⁷ Smac/DIABLO is a mitochondrial protein that is released upon apoptotic stimulation by ultraviolet irradiation and ionizing irradiation. It has also been shown that Smac/DIABLO binds to XIAP.⁶⁸ Researchers suggest that Smac/DIABLO and caspase-9 bind to overlapping sites on the BIR3 domain of XIAP. They also suggest that Smac/DIABLO acts by releasing caspase-9 from the XIAP/caspase-9 complex, leading to apoptosis activation. In contrast, survivin binds to Smac/DIABLO and inhibits its pro-apoptotic functions through releasing of XIAP from the Smac/DIABLO/XIAP complex to inhibit various caspases. In theory, C84A mutation in the extreme C-terminal region of the BIR domain can disrupt the Zn²⁺ coordination sphere and subsequently inhibit the interactions between survivin and Smac/DIABLO. Therefore, T34A/C84A survivin mutant may induce cancer cell death through two distinct mechanisms: (1) by forming a heterodimer with the wild-type survivin, leading to the reduced interaction with caspase-9; and (2) by reducing the interaction between survivin and Smac/DIABLO, leading to an increased binding between Smac/DIABLO and XIAP, resulting in the activity inhibition of XIAP.

Besides T34A and C84A survivin mutants, Song et al⁶⁹ showed a novel survivin mutant, Asp53→Ala (D53A), which had a function similar as T34A; several cellular functions of D53A were demonstrated. Asp53 is located within the BIR domain of survivin; it induced p53-independent apoptosis by forming a heterodimer with wild type survivin or forming

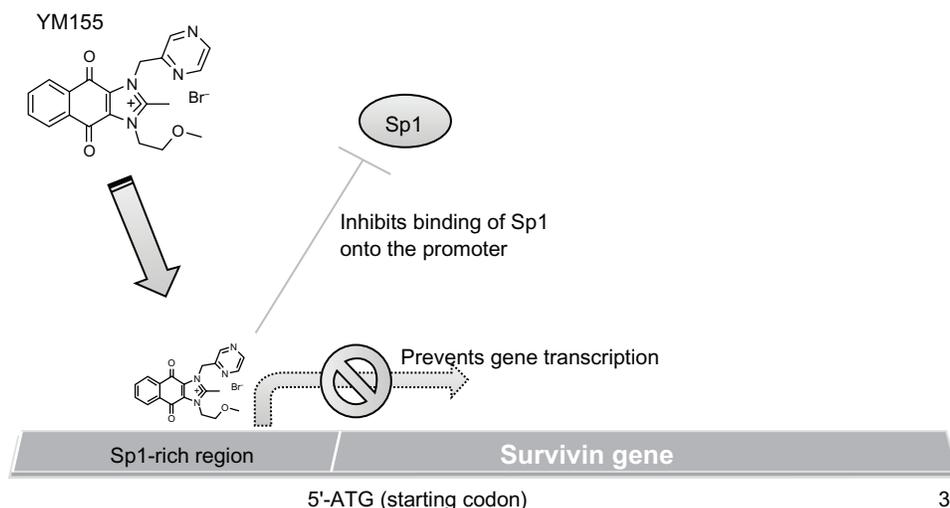


Figure 3 Chemical structure and functions of the survivin small molecule inhibitor, YM155. YM155 binds onto the specificity protein I (Sp1)-rich region of survivin promoter and inhibits survivin gene transcription.

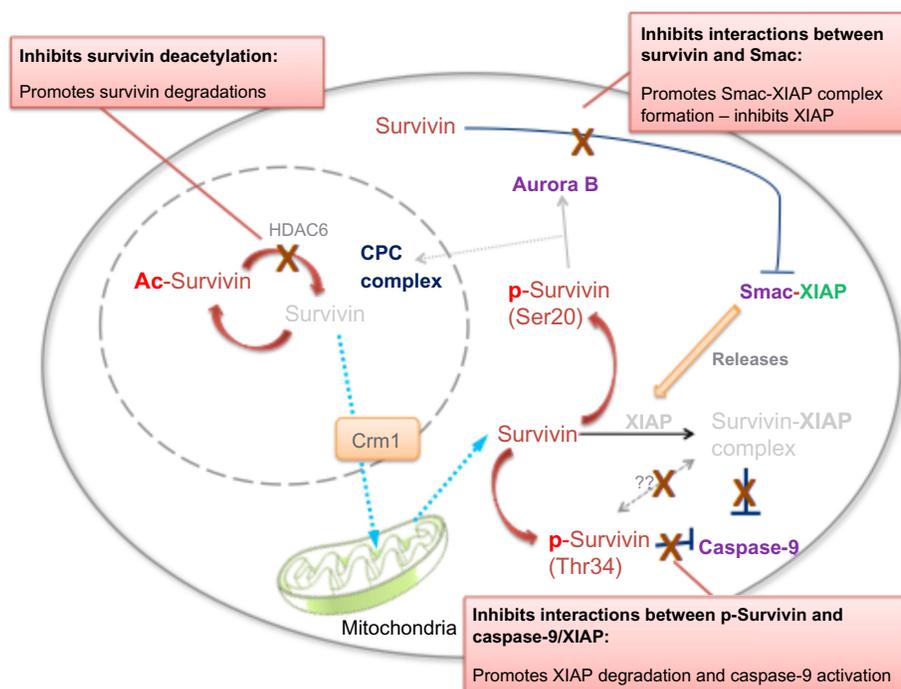


Figure 4 Future strategies of targeting survivin. Peptides or small molecule inhibitors can be developed to interfere with the interactions between survivin and its binding partners to either promote survivin degradation or to release the inhibition of various pro-apoptotic molecules.

Abbreviations: CPC, chromosomal passenger complex; STAT3, signal transducer and activator of transcription 3; Crm1, chromosome region maintenance 1; Smac, second mitochondria-derived activator of caspases; XIAP, X-linked inhibitor of apoptosis protein.

a homodimer to abolish anti-apoptotic activity of wild type survivin. In addition, the formation of both the homo- and heterodimer abolished binding to Smac/DIABLO.⁶⁹

Conclusion

Survivin plays multiple roles in the promotion of cancer cell survival. Surprisingly, only a few small molecule inhibitors were developed in the past, and only one of them, YM155 (survivin gene transcription inhibitor), has reached Phase II clinical trial (Figure 3). Although the development of survivin small molecule inhibitors has been limited in the past, recent advances in survivin biology do create opportunities for scientists to target survivin with various function-specific dominant-negative proteins/peptides/small molecule inhibitors that are capable of abolishing the interactions between survivin and its differential binding partners (Figure 4). Recent advances in nanotechnology also increase the possibility for using dominant-negative proteins, peptides or small interfering RNA as therapeutic agents in targeting survivin-positive cancer cells, given that application of nanoparticle encapsulation may enhance the stability of macromolecular drugs and further increase their functionality in vivo and in the human body.⁷⁰ Taken together, these scientific and technical advances open up new possibility for therapeutic

strategies that can be applied to target a specific function of survivin that is crucial for cancer cell survival, yet preserve functions of survivin that are important for normal cell survival.

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Disclosure

The authors declare that they have no competing interests.

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