# Is Survivin a new target for anti cancer therapy: Structural aspects, Molecular mechanism and Anti - Cancer Therapeutic strategies targeting Survivin - An Overview

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## Abstract

Survivin Protein is the smallest member of the Inhibitor of apoptosis protein (IAP) family. It is encoded by *BIRC5* gene consisting of 4 exons and 3 introns on chromosome 17q25. Survivin protein consists of 142 amino acid residues with one BIR domain in the amino terminal and helical region in the carboxyl terminal. Studies have observed that Survivin is a dual function IAP protein, it functions not only as a key inhibitor of apoptosis, binding to caspases 3,6 &7, the interaction disrupts the Caspase cascade of apoptosis, it is also found to be a critical regulator of the cell cycle. Survivin localizes to the mitotic spindle, where it interacts with tubulin and regulates mitosis. Survivin protein is expressed in embryonic and fetal tissues and in most of the human malignancies, it is however undetectable in differentiated tissues. This makes Survivin one of the distinctive IAP for targeted cancer therapy. And hence in this overview, efforts are made to focus on recent update on the advanced aspects of the key IAP protein Survivin with respect to its structural features, mechanism and therapeutic strategies specifically targeting the Survivin network.

**Key words:** Survivin, Apoptosis, Cell Cycle, Cancer, Target Therapy.

## Introduction

Cancer is a complex disease involving the multi- step process that comprises the metabolic and behavioral changes of the cells causing them to proliferate excessively in uncontrolled manner. The genetic and epigenetic factors also contribute to the disease process of cancer.[1]

Among the various molecular mechanisms contributing to cellular changes, the development of resistance to apoptosis is the major contributor to cancerogenic transformation. Resistance to apoptosis causes life span prolongation of the transformed cells, malignant progression and lack of tumor effective responsiveness to conventional modes of therapy such as surgery, chemotherapy, ionization and radiation therapies.[2,3,4]

Every cell has programmed for apoptosis, whereas, the apoptotic resistance is essentially contributed by structurally and functionally similar proteins called Inhibitor of apoptosis proteins (IAP).The IAP proteins are multifunctional proteins with one to three Baculovirus IAP repeat (BIR) domain, and a conserved zinc-coordinating Cys/His motif in the amino-terminus. The additional domains on the IAP proteins include Ubiquitin association domain and Ubiquitin conjugation domain these domains help in the proteasomal degradation and ubiquitination of specific caspases and suppression of apoptosis.[5,6]

Survivin belongs to the IAP protein family and is distinct from other IAPs by virtue of its expression seen in embryonic and fetal tissues and in most of the human malignancies, whereas it is undetectable in differentiated tissues.[7]

Studies have shown a higher expression of Survivin to be associated with tumor aggressiveness, angiogenesis and poor prognosis and alter the

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sensitivity of the tumor cells to chemotherapy. Therefore bio-molecules/compounds blocking the Survivin gene expression or targeting on action of the expressed protein can be an effective cancer therapeutic strategy.[7] Hence in this overview, efforts are made to focus on recent update on the advanced aspects of the key IAP protein survivin with respect to its structural features, mechanism and therapeutic strategies specifically targeting the Survivin network.

**Structure of Survivin and its Localization**

Of the eight members of the IAP proteins family, Survivin is a smallest member of the IAP Protein family containing a helical region in the carboxyl terminal and one BIR domain in the amino terminal. Survivin is a 16.5 kDa protein, dimeric in nature, structurally appears as bow- tie. It is expressed by *BIRC5* gene located on the telomeric end of the chromosome at loci 17q25.3. Structurally, the BIRC5 Gene contains 4 Exons spaced by 3 Introns, and translates to Wild type Survivin protein comprising 142 amino acid residues[8,9,10].

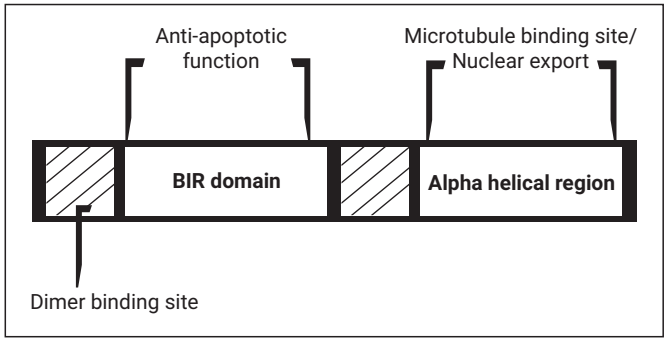


**Figure 1: Structure and function of Survivin protein**[10]

As Shown in Fig (1).The BIR domain of the intracellular cytosolic fraction of Survivin is important for anti-apoptotic function, the Alpha helix coiled region of the nuclear fraction of Survivin interacts with Microtubule structures and regulates the cell division. The Nuclear fraction of Survivin is claimed to control the cell division and the Cytoplasmic and Mitochondrial Survivin provides cytoprotection, the exact cellular localization of Survivin is controlled by nuclear export receptor crm1 present in the nucleus, which in turn is controlled by the Ran (**RA**s-related **N**uclear protein) GTPase activity. The Survivin/crm1 interaction is a critical factor in determining the bi-functional role of Survivin[10,11,12].

Survivin gene (14.7 kb), produce alternatively spliced transcripts such as wild type survivin, Survivin 2B with an additional exon (coding 165 amino acid protein), survivin 3B with five exons (coding 120 amino acid protein), Survivin 2$\alpha$ with 2 exons (coding74 amino acids), surviving 3$\alpha$ with two exons (coding 78 amino acids) and surviving ΔEx3 with deletion of exon 3 (coding 137 amino acid protein)[8,9,10]. Survivin isoforms also exhibit variations in their cellular localization, for instance the Survivin 2B is predominantly found in cytoplasm and Survivin ΔEx3 is found in the nucleus. But still the specific role of each splice variants in tumorigenesis, aggressiveness of cancer cells or resistance to therapy is unclear and yet to be explored.[11,12]

Research reports have demonstrated the biosynthesis of these isoforms in malignant cells to be at very high levels in comparison with normal tissues [13,14]. During cellular stress/ stimuli, the mitochondrial fraction of Survivin is postulated to be released in the Cytosol and binds to the SMAC (second mitochondria derived activator of caspase) and DIABLO (Direct IAP Binding protein with low pI proteins) proteins to suppress caspases activation.[15]

Few Studies have shown the extracellular fraction of Survivin secreted as membrane vesicles from the tumor cells stimulate the neighboring cancer cells to proliferate rapidly, with an increased potential to become invasive and to exhibit resistance to therapy[16,17].

**Molecular mechanisms of Survivin**

Research findings in yeast and Caenorhabditis elegans challenged the concept that Survivin is solely an apoptosis inhibitor protein, since it appeared to regulate cell cycle as well, thereby it is evident that, Survivin is a dual function protein, claimed to function as a key inhibitor of apoptosis as well as a critical regulator of the cell cycle.[18]

**Cell cycle and Survivin**

Regulation of cell division by the Survivin is argued as its predominant function. The tissue turnover of Survivin is observed to be regulated in a cell cycle dependent manner. Since the Survivin expression is highest during the G2/M phase and declines rapidly in the G1 phase of the cell cycle, it is effectively localized at different regions on the chromosomes during the cell cycle. During mitosis, Survivin localized to the mitotic spindle interacts with tubulin and regulates mitosis.[19] It tends to concentrate in the centromere in the G2 phase during the cell cycle, it then diffuses to the chromosome arms to be abundantly concentrated at the inner centromere during the prophase and metaphase of mitosis.[20] In anaphase, Survivin relocalizes at the central spindle[21]. At the end of telophase it is found to be associated with the mid body structure holding the two daughter cells together just before splitting into two separate cells[22].

Survivin forms a chromosomal passenger complex and binds with the target sites of centromere, mid plate and cleavage furrow, regulating proper chromosome segregation and cytogenesis[16].

Survivin here helps in targeting of the Chromosomal passenger proteins to the kinetochores, stabilizing the microtubules for the bipolar spindle formation[23]. Survivin specifically interacts with the proteins involved in cell cycle control mechanism like the CDK1 and CDK4, mitotic phosphorylation of Survivin by CDK1 promotes stability at metaphase[23].

Over expression of Survivin in cancer cells and its interaction with CDK4 has found to counteract G1 arrest of cell cycle and phosphorylation of tumor suppressor protein. The coordination of mitosis and cytokinesis is essentially a conserved role of Survivin in all eukaryotic cells[24,25]. The dysregulation of Survivin expression in human cancers evidenced due to epigenetic mechanisms by means of the hypo methylation at the promoter region of the *BIRC5* gene contributes to the high expression of Survivin in oral Squamous cell carcinoma.[26] Survivin expression levels was positively correlated with its promoter methylation in endometrial cancers results in the block of p53 binding and repressing of *BIRC5* gene leading to the over expression of Survivin.[27] Doxorubicin treated colon cancer HCT 116 cells showed the down regulation of BIRC5 transcription in these cells.[28]

**Survivin and apoptosis**

In a mammalian cell, the two mechanisms described of apoptosis are the intrinsic and the extrinsic pathway, both these pathways lead to the execution phase of apoptosis controlled by a group of proteins called caspases (**c**ysteine-**asp**artic acid prote**ase)**. Caspase 8 & Caspase 9 are the initiator caspases and Caspase 3, 6 and 7 are the executioner caspases. The executioner caspases induces the cleavage of protein kinase, cytoskeleton proteins, and activation of endonucleases, all of which contributes to the morphological changes in apoptosis.[29-32]

Cancer cells have adopted mechanisms that can resist apoptosis and promote cancer cell proliferation and survival. Though the mechanisms to resist programmed cell death by Survivin is sophisticated, it's largely witnessed in various studies that Survivin considerably contributes to the inhibition of apoptosis in cancer cells.[31]

The exact mechanism by which Survivin inhibits apoptosis is still unknown, however there are various reports that suggest the indirect and direct binding of Survivin to caspases 3,6 &7 disrupting the Caspase cascade and cleavage mediated by caspases, thereby causing reduced apoptosis. Few studies however have questioned the direct interaction of Survivin with caspases, this is due to the fact that Survivin does not possess a structural moiety that allows its direct binding to the effector Caspase 3, unlike the other IAPs.[33-35]

Another mechanism suggests that the Survivin prevents caspases activation by binding with the SMAC and DIABLO proteins released from mitochondria in the intrinsic pathway of apoptosis[33,34,35]. A study by Song et al. experimentally showed the interaction of Survivin with SMAC and DIABLO proteins and also observed that the blocking of this interaction triggered apoptosis in Taxol treated HeLa cells.[36] It is also postulated that Survivin in association with X-linked IAP (XIAP) inactivates the initiator Caspase-9[33,34].

Over expression of Survivin is reported in various human malignancy, as in Breast (90.2%), liver (87%), ovary (73.5%), bladder (57.8%), lung (85.5%), stomach (68%), esophageal (80%), oral (>75%) and hematological malignancies(68%).[11] Further from the above evidences, in the tumor cells with over expressed Survivin levels, Survivin can be claimed as a key role player in apoptosis regulation and molecules targeting Survivin can therefore be a potential target in cancer therapy.[11]

**Survivin Targeted strategies for Cancer Therapy**

Survivin targeted cancer therapy strategies, to improve the tumor response to apoptosis and inhibition of tumor growth can be broadly divided into the following approaches (1) Inhibition of Survivin transcription with Small interfering RNA (siRNA), ribozyme, and Antisense oligonucleotide (2) Post translational level Inhibition of Survivin (3) Immunotherapeutic approaches (4) Epigenetic strategies targeting Survivin for cancer treatment.[37-42]

**Inhibition of Survivin transcription**

The second generation antisense oligonucleotide drug LY2181308, small molecule Survivin suppressant Y155 in patients with advanced cancers has entered the phase II clinical trial, Y155 was shown to selectively inhibit the Survivin mRNA transcription and protein expression in various tumor cell lines.[42]

Ribozyme like $CUA_{110}$ (RZ7) and $GUC_{294}$ (RZ1) was transfected into human melanoma cell lines showed a reduced Survivin protein levels and increased caspase 9 dependent cell death, since the ribozymes are easily degraded they have not entered the clinical trials. The siRNAs which are short and double stranded inhibit the gene expression of Survivin. Survivin specific siRNAs transfected cancer cells show increased apoptosis and decreased cell growth

and proliferation. These siRNAs targeted at blocking Survivin-hsp90 association have demonstrated anti-cancer effects on androgen independent prostate cancer models.[37]

### Post translational level Inhibition of Survivin

CDK inhibitors like flavopiridol they counteract Survivin phosphorylation and accelerate Survivin degradation. Shepherdin is an antagonist of Survivin-Hsp90 complex, and is found to be highly critical in its anticancer activity.[38,39]

### Survivin based Immunotherapy

Immunotherapy with Survivin based anticancer vaccines has shown to suppress tumor growth in lymphomas, neuroblastoma, pancreatic and lung cancer, some of these efficacious vaccines have moved to the Phase II clinical trial. Vaccine approaches like dendritic cell based (DC) vaccines, DNA vaccines, peptide based vaccines for Survivin has been tested in the preclinical and clinical trials. A significant immunological response was also observed in Non-Small cell lung carcinoma (NSCLC) patients on a DC vaccine trial.[40,41]

### Epigenetic strategies targeting Survivin

A Short methylated oligonucleotide SurKex treatment of Non small cell lung cancer cell line NCI-H460 showed to induce site specific methylation on the BIRC5 promoter, the *BIRC5* mRNA and its coded protein Survivin were significantly reduced in NCI-H460 cells.[41]

Each type of cancer cells will exhibit different ways of regulating the *BIRC5* gene expression, understanding the type of epigenetic mechanism regulating *BIRC5* gene expression can result in novel epigenetic therapeutic approach towards cancer cell.[41]

### Conclusions

Survivin regulates cell division and apoptosis, and its concentration is undetectable in normal cells whereas over expressed in the human cancers, thus Survivin is recognized as a promising active target in cancer treatment and is under human cancer-clinical trials. But, there is a tremendous scope to have a drug or peptide that can control the function of Survivin as anticancer agent due to its specific role in cell division and apoptosis. Further research on epigenetic effects on the *BIRC5* gene in regulating the function of Survivin in cancer cells would be the future need, for holistic approach towards treatment of cancer patients targeting the molecule survivin.

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