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Targeting survivin in cancer: the cell-signalling perspective

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Survivin, a prominent anticancer target, is ubiquitously expressed in a plethora of cancers and the evolving complexity in functional regulation of survivin is yet to be deciphered. However, pertaining to the recent studies, therapeutic modulation of survivin is critically regulated by interaction with prominent cell-signalling pathways [HIF-1α, HSP90, PI3K/AKT, mTOR, ERK, tumour suppressor genes (p53, PTEN), oncogenes (Bcl-2, Ras)] and a wide range of growth factors (EGFR, VEGF, among others). In our article we discuss, in detail, an overview of the recent developments in the pharmacological modulation of survivin via cell-signalling paradigms and antisurvivin therapeutics, along with an outlook on therapeutic management of survivin in drug-resistant cancers.

Introduction

We, among others, reported that survivin, a bifunctional protein, is an inhibitor of apoptosis proteins, involved in cell division and apoptosis suppression [1–3]. It is a structural homodimer containing a single baculovirus inhibitor of apoptosis (IAP) repeat. Pharmacological regulation of the survivin gene is complex, involving multiple cell-signalling pathways at transcriptional and post-transcriptional levels. Transcriptionally, survivin expression is tuned by cell-cycle-dependent and -independent mechanisms [4]. During the post-transcriptional effects the survivin gene is therapeutically modulated by the stimulation of growth factors, such as protein kinases and insulin growth factor, that are mainly associated with the stabilization and translation mechanism of survivin messenger RNA (mRNA), relying on a mammalian target of rapamycin (mTOR) complex regulation that mediates cell growth in the phosphatidylinositol-3-kinase/serine/threonine protein kinase (PI3K/AKT) pathway [5,6]. Survivin is known to regulate apoptosis, cytokinesis followed by interaction with heat shock protein (HSP90), second mitochondria-derived activator of caspases (Smac/Diablo), Cyclin dependent kinase 4 (CDK4), cluster of differentiation 2 (CD2), Retinoblastoma /E2F complex, Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), p53 [3].

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Interestingly, hypoxia has a profound effect on survivin expression. We reported that increased survivin levels were exponentially correlated with hypoxia [3]. Survivin has a key regulatory role in the organization of chromosomal passenger complex through its interacting subunits [inner centromere protein (INCENP) and Borealin/Dasra B] that leads to the spindle apparatus formation during anaphase. Studies conducted at our laboratory successfully showed that interaction of aurora B kinase with survivin baculovirus IAP repeat (BIR) motif mutant can lead to accelerated G0/G1 growth phase arrested differentiated neuronal cells during mitosis [7].

This review will examine the state-of-the-art knowledge reporting the recent developments involved in therapeutic modulation of survivin via prominent cell-signalling pathways and antisurvivin therapeutics [dominant-negative survivin mutants, natural-product-derived therapeutics, nanodelivery systems and synthetic inhibitors (geldanamycin and derivatives, YM155, among others)]. In addition, pharmacological targeting of survivin in drug-resistant cancers is elaborated.

Therapeutic modulation of survivin through prominent cell-signalling pathways

Survivin is a nodal protein involved in multiple signalling mechanisms coordinating various cellular molecules, transcriptional networks and modifiers that directly or indirectly promote

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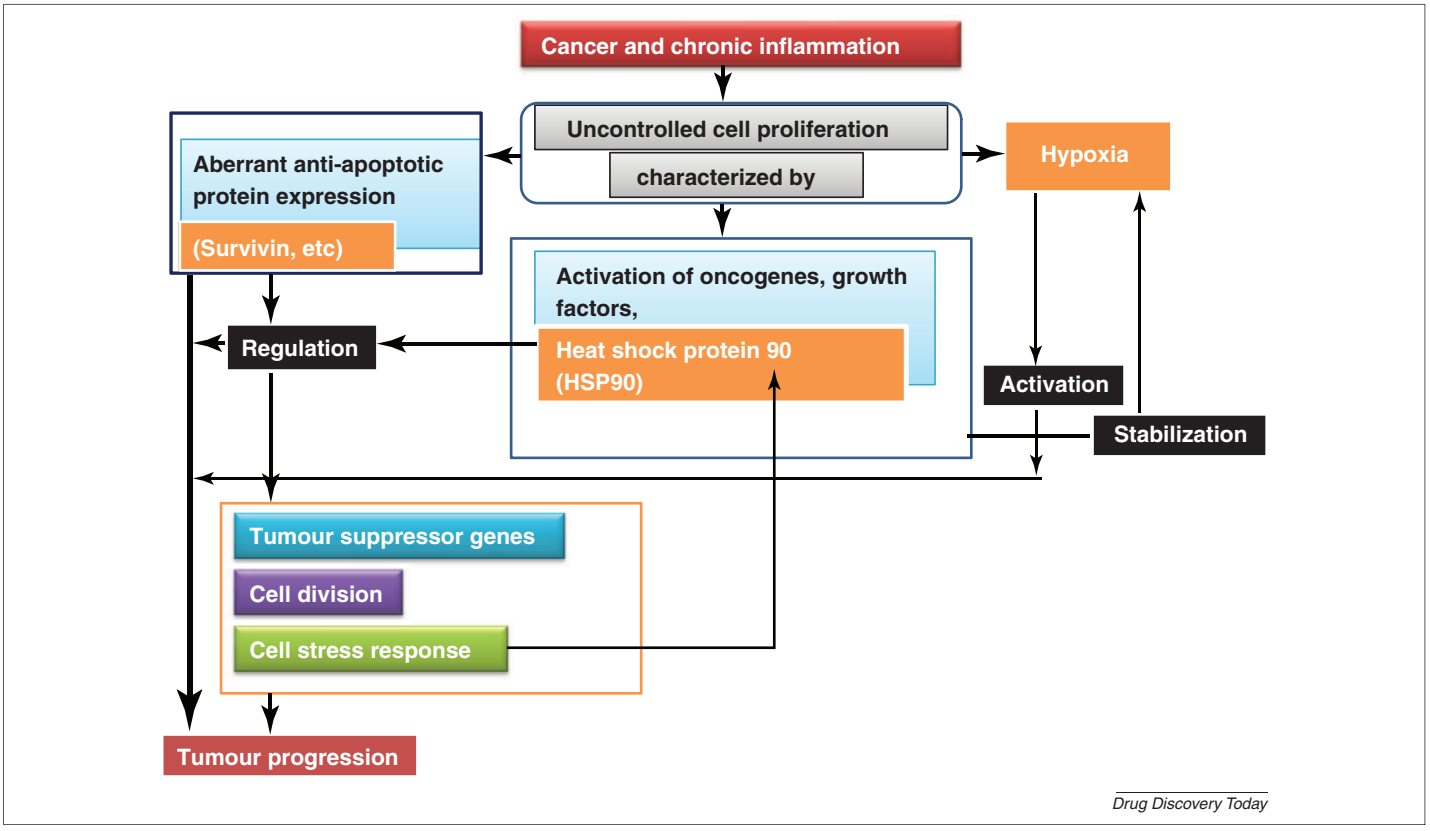

FIGURE 1

Flowchart demonstrating the interaction of survivin with prominent cell-signalling molecules. This flowchart outlines the interaction of survivin with cell-signalling molecules. Cancer and chronic inflammation are mainly characterized by uncontrolled cell proliferation, following aberrant expression of antiapoptotic proteins (e.g. survivin), activation of oncogenes, growth factors and heat shock protein 90 (e.g. HSP90) and hypoxia. Survivin promotes HSP-mediated tumour proliferation by the regulation of tumour suppressor genes, cell division and cell stress response, which in turn stabilizes hypoxia leading to activation of enhanced survivin expression, ultimately promoting tumour progression.

tumour proliferation by regulating cancer cell homeostasis [4]. We discuss the interaction of survivin with prominent cell-signalling pathways – HIF-1α and HSP90, the PI3K/AKT pathway, mTOR pathway, ERK pathway, tumour suppressors (p53, PTEN) and oncogenic (Ras, Bcl-2) signalling pathways (Figs 1,2; Table 1).

HIF-1α and HSP90

A positive correlation was observed in terms of increased HIF-1α and survivin expression, along with angiogenic factors, in a wide range of cancer cell lines (colon, pancreatic and breast cancer cell lines). Antisense oligonucleotides targeting HIF-1α caused marked downregulation of survivin in pancreatic cancer (BxPc-3 cell line) [8]. A transcriptional increase in survivin mRNA expression was observed in the presence of hypoxia and anticancer drugs in hepatocellular carcinoma [9]. Our laboratory studies conducted on HIF-1α and survivin expression in a combinatorial approach revealed effective inhibition of these molecules when targeted with antiangiogenic agents such as angiostatin or VEGF-blocking peptide, along with the antisense inhibition strategy that ultimately triggered efficient survivin downregulation causing inhibition of tumour angiogenesis [10,11].

HSP90 associated with survivin is overexpressed in cancers with roles in mitotic control and apoptosis inhibition. The cytoprotection mechanism of survivin–HSP90 association is centred on the mitochondrial pathway, where survivin has a role in the regulation of mitochondrial apoptosis specifically in tumours [12]. However, possible disruption of the survivin–HSP90 complex destabilizes survivin leading to mitochondrial apoptosis and ultimately cell growth suppression [12]. HSP90 interaction with survivin enables stabilization of cofactors such as AKT, human epidermal growth factor receptor (Erb-2) and HIF-1α, which can lead to tumour progression [13].

PI3K/AKT pathway

EGFR is known to regulate PI3K and ERK signalling in lung cancers. Experimental findings in the treatment of cancer cells with EGFR inhibitors caused inhibition of these pathways and triggered apoptosis [14]. The PI3K/AKT pathway is activated via EGFR signalling leading to upregulation of HIF-1α that transcriptionally activates survivin gene expression by binding to its promoter region [15,16]. Human epidermal growth factor receptor 2 (HER2)-mediated survivin upregulation in a breast cancer cell line revealed the roles of the PI3K/AKT pathway and herceptin. However, HER2-specific inhibitors efficiently downregulated survivin by inhibiting the PI3K/AKT pathway [17,18]. The functional mechanism involved HER2 overexpression leading to phosphorylation of 4EBP-1, a repressor of translation, allowing translational activation of survivin expression involving the PI3K/AKT pathway [19]. The PI3K/AKT pathway is attributed for its mechanism in inducing apoptotic resistance by increasing survivin expression

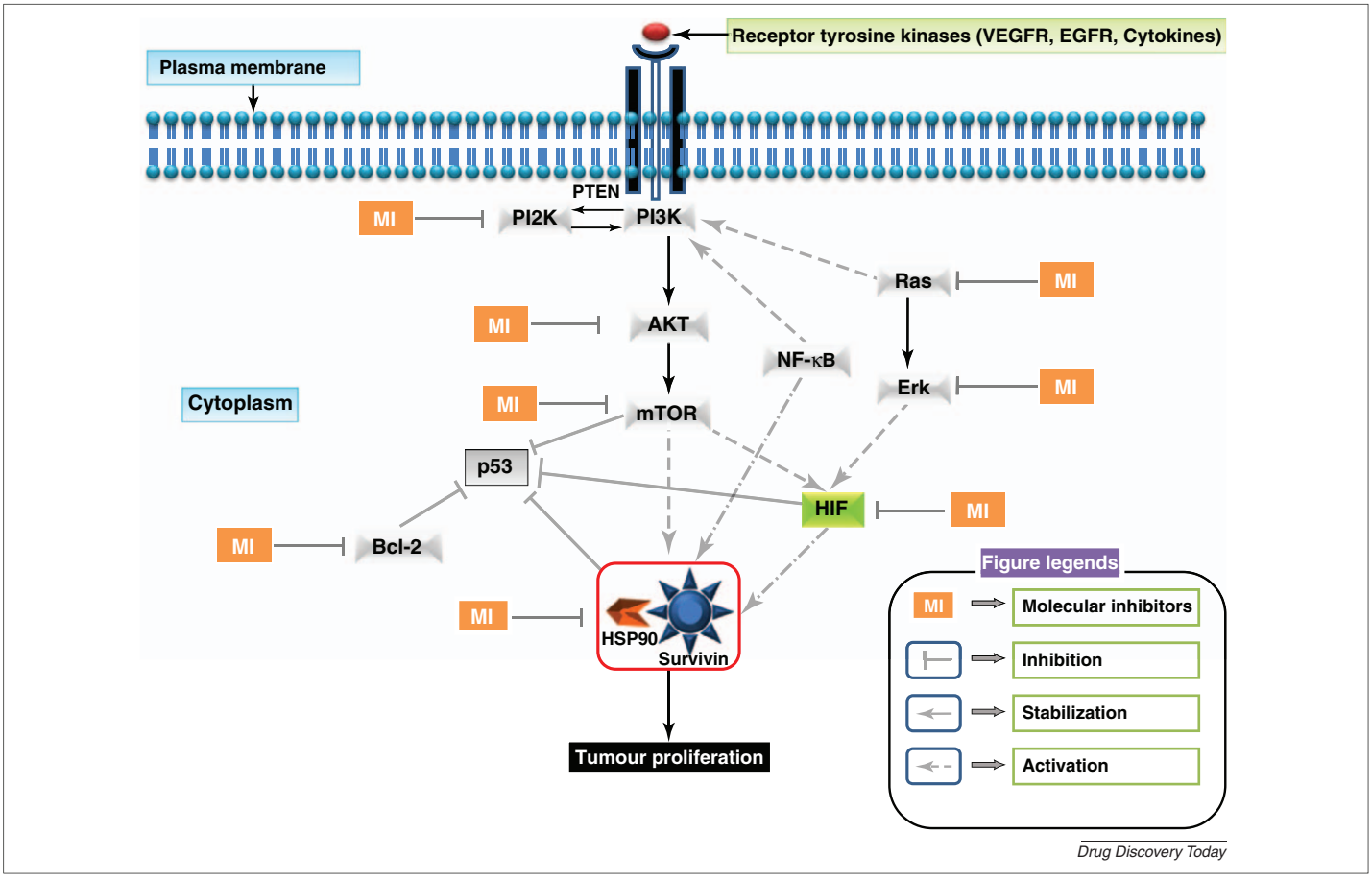

FIG. 2

Interaction of survivin with prominent cell-signalling molecules. The diagram illustrates the mechanism of interaction of survivin with cell-signalling molecules. Survivin–HSP90 complex is activated by the PI3K/AKT/mTOR pathway, further stabilized by NF-κB and HIF-1α, activated via the Ras/Erk pathway, leading to tumour proliferation. PTEN is deregulated and p53 functional inactivation is caused by mTOR, HIF-1α, Bcl-2 and also the survivin–HSP90 complex. Collectively, the above-mentioned events further stabilize the survivin–HSP90 complex promoting tumour proliferation. Molecular inhibitors targeting these key signalling molecules would be effective in promoting apoptosis by abrogating survivin expression and function.

[4], one such study conducted on head and neck squamous cell carcinoma and non-small-cell lung cancer cell lines proved that the PI3K/AKT pathway in combination with insulin-like growth factor-1 receptor (IGF-1R) caused apoptosis resistance to a farnesyltransferase inhibitor, SCH66336. Inhibition of AKT or knockdown of survivin expression subsequently induced apoptosis in response to this inhibitor [6].

mTOR pathway

Experimental studies conducted in non-small-cell lung cancer cell lines reported mTOR–AKT pathway-mediated survivin expression, leading to malignant progression due to tobacco carcinogens – nicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). However, siRNA knockdown of survivin expression suppressed tumour progression [20]. Studies conducted on pharmacological inhibition of mTOR by rapamycin in chronic lymphocytic leukaemia demonstrated mTOR-pathway-mediated survivin expression. Rapamycin induced G1/S cell cycle arrest in cell lines and subsequently caused downregulation of survivin. Similar results were obtained in a plasma cell myeloma cell line, leading to translational inhibition of survivin [21]. The mTOR complex in conjugation with survivin promotes chemoresistance in cisplatin-based therapy. Studies conducted on ovarian cancer cells showed downregulation of AKT by the drug triciribine. Short hairpin RNA (shRNA) transfection disrupted mTOR and survivin signalling, sensitizing cells to cisplatin [22]. mTOR in combination with IGF-1R modulates translational expression of survivin. Experimental studies conducted on prostate cancer cells proved that downregulation of mTOR by rapamycin and simultaneous suboptimal taxol treatments inhibited cell proliferation [5].

ERK pathway

Survivin is extensively expressed in acute myeloid leukaemia (AML) cells via the ERK pathway and haematopoietic cytokines. Experimental studies conducted on these cell lines with mitogen-activated protein kinase (MEK) inhibitor PD98059 demonstrated effective inhibition of the ERK pathway in normal AML cells and granulocyte macrophage colony-stimulating factor (GM-CSF) AML cells, suggesting the role of haematopoietic cytokine-mediated ERK signalling in regulating survivin expression at the mRNA and protein levels [23]. The ERK pathway in conjugation with AKT showed taxol-mediated survivin upregulation. Studies

TABLE 1

Inhibitors and modulators of survivin

Cell-signalling pathway	Inhibitor	Modulator	Refs
PI3K	Herceptin	EGFR, HIF, HER2	[15–18]
PI3K	SCH66336	FSH, VEGF, Haematopoietic cytokines, Id1	[6,23,78,79]
mTOR	Nicotine and NNK	IGFR1	[5,20]
mTOR	Triciribine	Ras	[22,80]
ERK	PD98059, imatinib, quercetin	Bcr-Abl	[23,26,31]
p53	Decitabine	E2F-1, APC, Hypoxia, p300/CBP, Ran-GTP	[4,28,81–83]
PTEN		FOXO1, FOXO3a	[29]
Bcl-2, NF-κB	Curcumin, ABT737	p53, p38 MAPK	[32,84,35]
Bcl-2	TW37, 15d-PGJ2 and TGZ, STAT3	PPAR-g	[33,34,85]
Ras	Lovastatin	PI3K, MEK, MAPK	[36,86,87]
Ras, ERK	FTS (salirasib)	E2A-HLF	[88,37]

Cell-signalling pathway (CSP): PI3K – phosphoinositide 3-kinases, mTOR – mammalian target of rapamycin, ERK – extracellular signal-regulated kinases, p53, PTEN(Phosphatase and tensin homolog) – tumour suppressor proteins, Bcl-2,Ras – oncogenes, NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells. Inhibitor: SCH66336 – farnesyltransferase inhibitor, NNK – (4-methylnitrosamino)-1-(3-pyridyl)-1-butanone), PD98059 – mitogen-activated protein kinase (MEK) inhibitor, ABT-737 – molecular inhibitor, TW-37 – molecular inhibitor, (15d-PGJ2) – (15-deoxy-delta(12,14)-prostaglandin J2), TGZ – troglitazone, STAT3 – signal transducer and activator of transcription, FTS: salirasib – (S-farnesylthiosalicylic acid), lovastatin – (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (HRIs)). Modulator: EGFR – epidermal growth factor receptor, HIF – hypoxia inducible factor, HER2 – human epidermal growth factor receptor 2, FSH – follicle-stimulating hormone, VEGF – vascular endothelial growth factor, Id1 – inhibitor of differentiation, IGFR1 – insulin growth factor receptor-1, Bcr-abl – oncogene fusion protein, E2F-1 – transcription factor, APC – adenomatous polyposis coli protein, p300/CBP – transcriptional co-activator proteins, Ran–GTP pathway – Ran (Ras-related nuclear protein)–guanosine-5′-triphosphate pathway, FOXO1 and FOXO3a – transcription factors, p38 MAPK – P38 mitogen-activated protein kinases, (PPAR-g) – peroxisome proliferator activated receptor-g ligands, E2A-HLF – transcription factor.

conducted on taxol-resistant cell lines proved that a *cis*-acting DNA element upstream of the 1430 survivin promoter region was responsible for survivin regulation during taxol treatment. Antisense knockdown of survivin in combination with pharmacological inhibition of AKT and ERK signalling increased taxol-induced cell death [24]. Ras-dependent ERK pathway activation induced survivin expression in a human glioblastoma cell line. Studies conducted using the Ras inhibitor farnesylthiosalicylic acid (FTS) caused efficient downregulation of Ras-dependent ERK pathway activation leading to decreased survivin levels [25]. Investigations on prostate cancer cells for survivin expression by quercetin caused tumour necrosis factor related apoptosis-inducing ligand (TRAIL)-induced apoptosis. Experimental findings suggest that quercetin promoted ERK-mediated deacetylation of the histone H-3 region of SP1, a transcription factor that binds to the survivin promoter. This leads to downregulation of survivin by inhibiting SP1 binding activity, simultaneously enhancing TRAIL cytotoxicity [26].

Tumour suppressor genes

p53

Transcriptional inhibition of survivin was mediated via stabilization of p53 by doxorubicin. Stabilized p53 proteins bind to the p53-binding site in the survivin promoter region, hence suppressing its activity via drug *trans*-acting mechanisms. However, studies on survivin promoter activity in terms of sequence-selective DNA-binding anticancer agents that can modulate survivin expression and also their role in drug mechanisms can lead to novel incentives to observe survivin transcription in anticancer strategies [27]. Increased expression of survivin in endometrial tumours was correlated with hypermethylation that prevented binding of p53 and PTEN causing inactivation of DNA repair enzymes and O6-methylguanine DNA methyltransferase (MGMT). Later, demethylation of the survivin promoter by decitabine resulted in p53-dependent transcriptional survivin repression. Results suggest that demethylation induced by a chemotherapeutic agent is active in cancers by allowing the functional reactivation of tumour suppressor genes such as p53, among others. These findings validated with gel shift assays show that DNA methylation based approaches to trigger p53-dependent apoptosis and survivin repression are highly tissue/context specific [28].

PTEN

PTEN directly decreases survivin via FOXO1 and FOXO3a transcription factors in the survivin promoter region. *In vivo* and *in vitro* studies in prostate cancer cells demonstrated that PTEN tumour suppression was inversely correlated with survivin expression. Hence, it was suggested that disruption of constitutive expression of survivin is essential for the function of endogenous PTEN tumour suppression [29]. Studies conducted in endometrial adenocarcinoma revealed increased survivin expression along with decreased activity of the PTEN gene. However, a correlation was not established between survivin and PTEN, which suggest that survivin exerts in proliferative activity by different mechanisms in endometrial cancer development [30].

Oncogenes

Bcl-2 oncogene

Quercetin, a bioflavonoid, was investigated for its antiproliferative effects in the human liver carcinoma cell line (HepG2) and induced apoptosis in a dose- and time-dependent manner. The mechanism of quercetin-induced apoptosis is mediated by the downregulation of survivin and Bcl-2 protein expression, possibly by a p53-independent manner directly regulated by p53 or by G2/M cell cycle arrest dependent regulation of survivin [31]. The pharmacological inhibition profile of curcumin was examined for its mechanism in promoting apoptosis in ovarian carcinoma cells. Curcumin exhibited cytotoxic activity in ovarian cancer cells

TABLE 2

Gene-therapy-based nanodelivery systems for survivin

Nanodelivery system	Sequence	Mechanism of action	Key molecules	Study model	Refs
Polyamidoamine (PAMAM) dendrimer-conjugated MPNs antisurvivin oligonucleotides (asODN)	siRNA-(5′-CCCAGCCTTCCAGCTCCTTG-3′)	Blocking translation of survivin mRNA	Ribosome, survivin	*In vitro*	[59–62]
LPD (liposome polycation-DNA) PEGylated nanoparticle coupled with anisamide, conjugated to survivin siRNA	siRNA 5′-GGCUGGCUUCAUCCACUGCdTdT-3′; 3′-dTdTCCGACCGAAGUAGGUGACG-5′	Sigma receptor-mediated pathway	Sigma receptors, survivin	*In vitro*	[54,61,63]
Antisurvivin amino silica nanoparticles (NH_2SiNPs)	siRNA (5′-V-TGTGCTATTCTGTGAATT-3′-V)	Antisense inhibition of survivin	Survivin	*In vitro*	[66]
(Locked nucleic acid) LNA oligonucleotide nanoparticle	siRNA CCCAGCCTTCCAGCTCCTTG(OEG)SH	Inhibiting gene expression	Survivin	*In vitro*	[67]
Magnetic iron oxide nanoparticles coupled with membrane translocation peptide conjugated to survivin siRNA	Translocation peptide – [Myr-Ala-(Arg)7-Cys-$CONH_2$]	Enhanced apoptosis and necrosis	Survivin	*In vivo* (mouse	[50,68,69]
Antisense survivin plus liposome	Sense primer (5′-GAGTCGTCTTGGCGGAGG-3′) Antisense primer (5′-CTTAGATGTGGCATGTCACTC-3′)	Antisense inhibition of survivin	Survivin	*In vivo* (mouse	[3]
Dominant-negative survivin plus liposome	Primers C84AF (5′-CACTCCCCGGGCGCAGCCTTCCTCAC-3′) C84AR (5′-GGCTGCGCCCGGGGAGTGCTT-3′)	Blocking endogenous survivin function by competitive binding to survivin effectors	Survivin	*In vivo* (mouse models)	[3]
Combination of antisense plus dominant–negative survivin and liposome	Antisense strategy Sense primer (5′-GAGTCGTCTTGGCGGAGG-3′) Antisense primer (5′-CTTAGATGTGGCATGTCACTC-3′) Dominant-negative survivin primers C84AF (5′-CACTCCCCGGGCGCAGCCTTCCTCAC-3′) C84AR (5′-GGCTGCGCCCGGGGAGTGCTT-3′)	Antisense inhibition and competitive binding to survivin effectors	Survivin	*In vivo* (mouse models)	[3]

by a p53-independent pathway involving p38 mitogen-activated protein kinase (MAPK) activation, followed by the ablation of AKT signalling and leading to decreased expression of antiapoptotic proteins – Bcl-2 and survivin [32]. Studies were conducted to decipher the novel mechanism of action by peroxisome proliferator activated receptor-g (PPAR-g)-ligand-induced apoptosis on myeloid leukaemia K562 and HL-60 cell lines. 15-Deoxy-delta(12,14)-prostaglandin J2 (15d-PGJ2) and troglitazone (TGZ) molecular antagonists were examined for their apoptotic inducing mechanisms leading to upregulation of bax; moreover, the effective downregulation of survivin and Bcl-2 expression was observed. Therefore, these findings recommend PPAR-g ligands as potential therapeutic targets in myeloid leukaemia cells [33]. Studies were conducted using TW37, a small-molecule inhibitor of the Bcl-2 family of proteins in pancreatic cancer cells. The mode of action of TW37 involved inhibition of cell cycle related genes leading to S-phase cell cycle arrest causing survivin downregulation [34]. Investigations were conducted using ABT737, a molecular inhibitor of the Bcl family of proteins in a refractory Hodgkin lymphoma (HL) cell line. This molecule exhibited concentration- and time-dependent cytotoxicity with possible mechanisms involving downregulation of Bcl-2, Bcl-xL, survivin protein and also NF-κB. Hence, these results provide a background to improve the pharmacological profile of the inhibitor further [35].

Ras oncogene

Investigations were carried out on SW480 (a colon cancer cell line) using lovastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (HRI), which blocks the cholesterol synthesis pathway leading to survivin downregulation and promoting apoptosis. The mechanism of action of lovastatin involved inhibition of Ras-mediated PI3K activation via prevention of Ras isoprenylation, a mechanism that activates the biological function of Ras proteins [36]. Studies were conducted on human tumour cells using the Ras inhibitor FTS (salirasib) that reregulates Ras pathways by the suppression of survivin expression and transcription factors – stress responses that control aberrant cell proliferation. FTS caused transcriptional suppression of survivin. Therefore, it can be noted that FTS can be a prominent treatment regime for Ras-deregulated tumours [37]. Similar studies were conducted using FTS in U87 glioblastoma multiforme cell lines and results were in accord with previous studies as discussed. However, forced expression of survivin resulted in FTS functional inhibition restoring Ras tumour pathways [25].

Antisurvivin therapeutics

A wide spectrum of molecular inhibitors are available to counteract survivin expression and function for decreasing tumour growth potential leading to enhanced tumour cell apoptotic response to the targeted drug molecules. In this section we discuss prominent therapeutic strategies employed in targeting survivin [38,39] (Tables 1 and 2).

Dominant-negative survivin mutants and gene therapy

We developed a recombinant cell-permeable dominant-negative survivin molecule fused with a poly-arginine (R9) cell-penetration peptide domain forming a complex - SurR9–C84A. This construct successfully penetrated tumour cells abrogating survivin function and thereby causing caspase-independent and -dependent apoptosis in cancer cells. Furthermore, we discovered that SurR9–C84A could activate TNF-α signalling in 3D prostate and cervical cancer cell models, followed by marked downregulation of survivin expression [2]. Our laboratory studies in development of the dominant-negative mutant of survivin (SurR9–C84A) revealed its neuroprotective ability as having a proliferative effect in the differentiated neuronal cell lines SK-N-SH and HCN-2, primarily mediated by its regulatory mechanisms in modulating microtubule binding and stability during mitosis. The neuronal cell lines differentiated in the presence of retinoic acid exhibited cell proliferation in a dose-dependent manner, when treated with SurR9–C84A, causing decreased expression of prominent neuronal cell death markers – caspase 3 and cyclin D molecules – thereby leading to a decline in neuronal cell death. Furthermore, we examined the neuroprotective role of SurR9–C84A in SK-N-SH cells against granzyme B (GrB) which exhibits cytotoxicity with the help of activated T cells. The functional mechanism of action of pretreated SurR9–C84A SK-N-SH cells involved the maintenance of cytosolic Ca^{2+} homeostasis causing decreased mitochondrial membrane depolarization followed by the regression of cyclin D and caspase 3 expression, ultimately defying the apoptosis mechanism. Similar observations were noted in H_2O_2-pretreated SK-N-SH cells against oxidative stress, where SurR9–C84A could confer neuroprotective ability by the regression of mitochondrial depolarization, further maintaining a repressive effect on cyclin D, caspase 9 and caspase 3 expression [40].

Hence, we hypothesize the dual selective ability of SurR9–C84A in inducing apoptosis and also that the neuroprotective effect can lead to new therapeutic management of cancers and neurodegenerative diseases [41–43]. B7-1, an important factor that is expressed on antigen presenting cells (APCs), interacts with CD28 cells and thereby promotes a T-cell-mediated immune response against cancer cell growth. We conducted studies to decipher the role of survivin in enhancing the immunosuppressive effect in tumours by prompting immune resistance to cytotoxic T cells (CTLs). *In vivo* mouse studies were conducted with a combinatorial treatment approach involving B7-1 in association with dominant-negative survivin or antisense survivin and results obtained showed marked downregulation of survivin causing increased production of CTLs, further leading to growth inhibition. Thus, investigations prove, for the first time, that survivin expression in relation to B7-1 is a promising diagnostic and prognostic marker for therapeutic manipulation of tumours [3].

Natural-product-derived compounds

Curcumin, a potent natural compound with anticancer activity is now being widely studied to decipher its anticancer mechanisms. *In vivo* pancreatic cancer cell model investigations revealed the inhibitory effect of curcumin on SP transcription factors causing functional inactivation of the NF-κB pathway, leading to regression of downstream targets – survivin, VEGF and cyclin D molecules – along with posing a decrease in mitochondrial membrane potential unveiling its mitochondriotoxic effect [44]. The mitochondriotoxic effect of curcumin is mainly due to functional activation of caspases 8, 9 and 3, PARP cleavage and BH3-interacting domain death agonist (BID) domain truncation causing release of cytochrome *c*, and downregulation of factors such as survivin, AKT, FOXO and

GSK3 – ultimately leading to mitochondrial apoptosis [45]. Further clinical trials with curcumin on cutaneous T-cell lymphoma (CTCL) patients confirmed exquisite regression of survivin and Bcl-2 levels, caused due to functional inactivation of STAT3 and NF-κB signalling mechanisms followed by caspase activation induced apoptosis [46]. Limonoids derived from *Azadirachta indica* (Neem), were explored for their anticancer activity on Hela cells. Research findings confirmed functional ablation of survivin, cyclin B, cyclinD1 and PCNA molecules leading to GO/G1cellcycle arrest in a dose dependent manner. Furthermore, coherent decrease in mitochondrial potential was noted that effectively shunted NF-κB and caspase2 signalling mechanisms having a retrogradatory effect on survivin [47]. However, further *in vivo* mouse studies undertaken in an oral cancer cell model to decipher the inhibitory effect of limonoids on survivin expression demonstrated an increase in nuclear survivin but a decrease of its cytoplasmic counterpart [48]. Studies conducted on pancreatic cancer cell lines showed chemoresistant behaviour to gemcitabine treatment followed by enhanced survivin expression. Emodin, a medicinal herb, was used in combinatorial therapy with gemcitabine to suppress cancer cells by the downregulation of survivin and also β-catenin, which is known to interact with survivin promoting chemoresistance [49]. Recently we have shown the downregulation of survivin expression in colon cancer cells (Caco-2 and HT-29) after treatment with bovine lactoferrin, a multifunctional natural protein.

Nanodelivery systems

Nanoscale devices, when coupled with functional biological molecules such as receptor-mediated tumour-specific ligands, antibodies, anticancer drug combinations, drug encapsulations and also imaging probes, are much smaller than cancer cells and can be used for targeted drug delivery with enhanced selectivity [50–53]. siRNA based therapeutic targeting of survivin is a well established strategy with possible limitations of nonspecific delivery and degradation of naked siRNA by cell endo, exo-nucleases and immune stimulation [54]. Hence, nanotechnology-based strategies are evolving to comprehend these issues and enable a targeted therapeutic approach (Table 2).

Our research studies undertaken in human colon cancer cells (Caco-2 and HT-29) to assess the activity of alginate-coated nanocarriers (ACNC) loaded with covalently cross-linked CPP (R9 and Tat peptides) complexed with antisense survivin exhibited enhanced antitumour activity with increased bioavailability and sustained release of the drug molecules [55,56]. Furthermore, investigations carried out in human breast cancer cells (MDA-MB-231 and MCF-7) with alginate gel-encapsulated chitosan ceramic nanocore nanocarriers (ACNC-NPs) loaded with DNSurR9 and shepherdin, along with oncogenic antisense miRNA-27a, caused marked downregulation of survivin along with angiogenic factors such as VEGF receptor. The mechanism of action involved promotion of apoptosis by enhanced antisense activity followed by

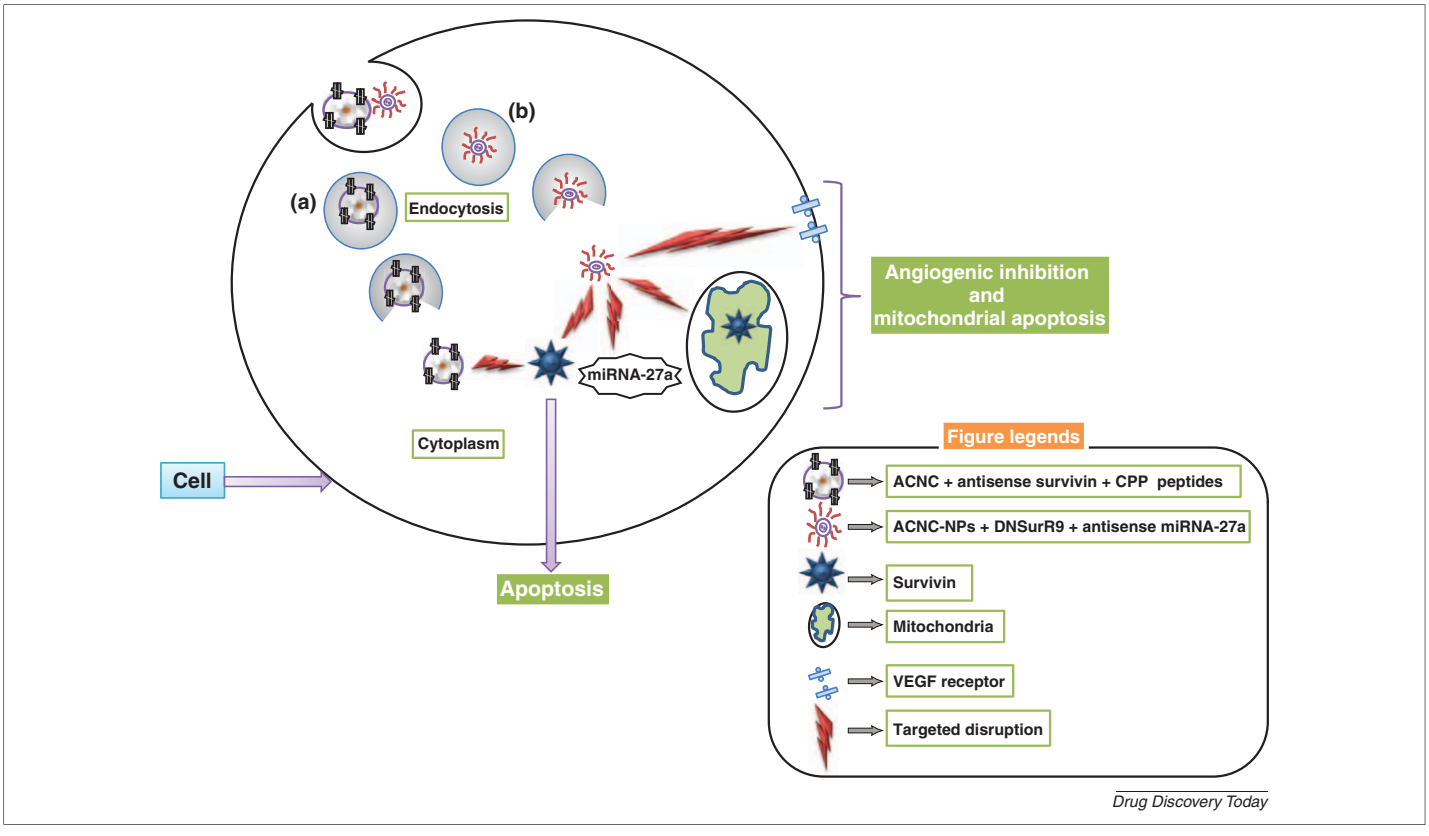

FIG. 3

Nanotechnology-mediated drug delivery for survivin. This figure illustrates compartmentalized oriented nanotechnology-based drug delivery to survivin. Intracellular uptake of **(a)** alginate-coated nanocarriers (ACNC), nanocarriers complexed with antisense survivin and cross-linked CPP (R9 and Tat peptides); and **(b)** alginate gel-encapsulated chitosan ceramic nanocore nanocarriers (ACNC-NPs), loaded with (DNSurR9) and complexed with antisense miRNA-27a, are mediated by endocytosis. The ACNC antisense survivin CPP peptide delivery system counteracts the action of cytosolic survivin triggering apoptosis. The ACNC-NP–DNSurR9 antisense miRNA-27a delivery system exhibited multifunctional nature inhibiting cytosolic survivin, oncogenic miRNA-27a, VEGF receptor and caused disintegration of mitochondria leading to angiogenic inhibition and mitochondrial apoptosis.

disintegration of mitochondrial compartments in cancer cells. Hence, we illustrate a novel therapeutic mechanism with our nanodelivery system which can be placed in the radar of pharmacological inhibition strategy for antisurvivin treatments [2,7,56–58] (Fig. 3).

Polyamidoamine (PAMAM) dendrimer-conjugated MPNs were designed to deliver antisense survivin oligonucleotides (asODNs) to tumour cells. The nanoparticulate system, designed with more positive charges on the surface, was highly internalized across the cell membrane and had a profound inhibitory effect on the survivin gene and protein expression [59–62]. Liposome polycation (LPD) DNA nanoparticle formulation with anisamide, as a component that binds specifically to sigma receptors that are highly expressed in lung cancer cells, was developed by Li and Huang for specific delivery of survivin siRNA. *In vitro* studies demonstrated improved gene delivery efficiency suppressing survivin expression, thereby sensitizing cancer cells to the chemotherapeutic drug cisplatin and promoting apoptosis [54,61,63,64]. A similar nanoparticle system was developed, with additional PEGylated lipid tethered to the ligand, that improves nanoparticle stabilization. *In vitro* studies revealed greater transfection efficiency with improved downregulation of survivin mRNA and protein, decreased cellular proliferation and enhanced sensitization of cancer cells to anticancer drugs [64,65]. Investigations using antisurvivin amino silica nanoparticles (NH_2SiNPs) proved enhanced gene delivery efficiency in Hela and epithelial carcinoma cells (A549), inhibiting survivin expression and cell proliferation. Furthermore, the results, when compared with the liposomal delivery system antisurvivin oligonucleotide (NH_2SiNPs), were more biocompatible and exhibited zero cytotoxicity with more-improved transfection [66]. Locked nucleic acid (LNA) oligonucleotide nanoparticle (LNP) conjugates were developed by utilization of thiol-linked gold nanoparticles terminated with locked nucleic acid oligonucleotides. This complex formed stable duplexes causing effective binding with the complementary sequences in the cells that triggered gene silencing. In this study, LNA–antisurvivin–oligonucleotide–NP conjugates were incubated with lung carcinoma A549 cells and survivin expression was examined. Results suggested that LNPs significantly downregulated survivin protein levels in a dose dependent manner as confirmed by western blot analysis, in comparison with control samples [67]. Dual purpose magnetic iron oxide nanoparticles were designed for *in vivo* targeting of survivin expression in mouse tumour models. These magnetic nanoparticles were covalently linked to survivin siRNA for therapeutic targeting and simultaneously labelled with an infrared (IR) dye to enable near-IR *in vivo* optical imaging and high resolution magnetic resonance imaging (MRI). Furthermore, these nanoparticles were also coupled with a membrane translocation peptide for efficient intracellular delivery. Experimental results reported drastic inhibition of survivin, verified by RT-PCR, leading to enhanced tumour apoptosis and necrosis compared with controls [50,68,69].

Synthetic inhibitors

Geldanamycin and derivatives

Exposure of a sarcoma cell line to 17-AAG caused downregulation of survivin leading to enhanced apoptotic response and also radiosensitization [70]. Investigations conducted on Hela cells demonstrated loss of survivin expression in a dose-dependent manner with geldanamycin (GA), with growth arrest at G2/M phase transition with consistent inactivation of the p53–Rb complex [12]. Studies conducted at our laboratory showed that geldanamycin- and 17-AAG-treated human cancer cell lines (A549, HONE-1 and HT-29) demonstrated an increase in survivin expression at the translational level and, therefore, we hypothesize that HSP90 inhibitors regulate survivin expression at different stages; but further studies need to be conducted to decipher the exact mechanism of action of these inhibitors on survivin [13].

*1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1*H*-naphtho[2,3-d]imidazolium bromide (YM155)*

Gene reporter assays were performed with transformed cells expressing a stable survivin gene promoter region, driven by the luciferase reporter gene, to screen for potent inhibitors of the survivin promoter. An imidazolium-based compound known as YM155 seemed to be targeting survivin expression effectively at low subnanomolar concentrations. YM155 exhibited its activity *in vitro* and *in vivo* by escalating caspase-mediated apoptotic mechanisms and inhibition of DNA repair with increased radiosensitivity by the downregulation of survivin in a time-dependent manner [71], which in turn can sensitize the tumour cells to conventional chemotherapeutic strategies [72]. Investigations undertaken to evaluate the effect of YM155 on various antiapoptotic proteins showed reduction in survivin and conversely no effect on c-IAP2, XIAP, Bcl-2, Bcl-xL, Bad, α-actin and h-tubulin proteins, mitotic mechanisms, G2/M phase cell cycle checkpoint and alteration of the phosphorylated form of AKT – showing differential selectivity of YM155 on survivin expression independent of functional p53 gene status in tumours [71]. Preclinical studies have proved the activity of YM155 in suppressing survivin expression at the protein and mRNA levels. In addition to this, pharmacokinetic analysis showed that profound concentrations of YM155 were present in tumour tissue when compared with plasma [73]. Elucidation of YM155 activity and the cell signalling pathways involved, in combination with conventional radio- and chemo-therapeutic treatment platforms, can provide a rationale for anticancer therapies [72].

Pharmacological targeting of survivin in drug-resistant cancers

Survivin promotes chemoresistance by stabilizing microtubule organization. Studies conducted using BPR0L075, a tubulin-depolymerizing agent, on a BPR0L075-resistant KB cell line showed enhanced survivin expression. Therefore, conditional deletion of the survivin gene with a specific siRNA molecule markedly induced sensitivity for BPR0L075. Hence, results suggest that administration of a microtubule-destabilizing agent in combination with a survivin inhibitor can prove worthwhile for the treatment of chemoresistant cancers [74]. Research studies were carried out using T138067, a compound active against multi-drug-resistant tumour cell lines. However, owing to the induction of survivin expression via the PI3K/AKT and MEK/ERK pathways, the drug had a less pronounced apoptotic effect on chemoresistant tumours. Furthermore, a new molecule, SN8, metabolite of irinotecan known to inhibit survivin, was used in combination with

BOX 1

Drug delivery approach

Target centric – direct delivery to the targeted entity.
Pathway oriented – delivery to nodal proteins that can affect multiple targeted entities.
Compartmentalized pathway oriented – delivery to nodal proteins that can affect multiple targeted entities located in intracellular organelles.

T138067 and, interestingly, this drug formulation effectively inhibited survivin expression promoting apoptosis [75]. Research findings were reported that an ErbB2-mediated transcriptional increase in survivin expression causes taxol resistance in breast cancer cell lines. Downregulation of the survivin gene by a specific siRNA molecule sensitized cells to taxol treatment. PI3K/AKT and sarcoma (Src) gene activation promotes a translational increase of survivin expression via the mTOR/eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) pathway. Therefore, inhibitors acting on ErbB2 and the PI3K/AKT pathway can help in modulating taxol resistance in terms of survivin expression [19]. Research studies were carried out using nucleoside analogues, TAS106 (3′-C-ethynylcytidine) in radioresistant tumour cells. TAS106, used in combination with HIF-1α, in hypoxic tumour cells caused IR-radiation-induced apoptosis, subsequently downregulating survivin expression. Furthermore, TAS106 has reached Phase I/II clinical trials in anticancer therapies [76].

Concluding remarks and future directions

In conclusion, understanding the pharmacology of survivin in cancer biology can certainly lead to the development of sustainable therapeutic approaches. Recent studies were undertaken to evaluate the subcellular localization and functional aspects in the mitochondrial signalling network, and the ambiguous behaviour of survivin interactions with molecular inhibitors is a topic of current debate that is still under consideration [77]. However, the evolving complexity in survivin signalling mechanisms has retrograded the current therapeutics from specifically targeting survivin. Adhering to the knowledge generated from our research studies, we illustrate a novel nanotechnology-based paradigm that can selectively target subcellular survivin localization and trigger survivin downregulation – when utilizing biocompatible natural-product-derived therapeutics with RNAi, microRNA and aptamer-enabled technologies [55–57] (Fig. 3; Box 1). The treatment strategies mentioned in this review detect and oversee survivin expression ultimately leading to the inhibition of survivin.

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