Targeting survivin in cancer therapy: fulfilled promises and open questions

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Survivin is a bifunctional protein that acts as a suppressor of apoptosis and plays a central role in cell division. The protein is strongly expressed in the most common human neoplasms, has prognostic relevance for some of them and appears to be involved in tumor cell resistance to anticancer agents and ionizing radiation. On the basis of these findings, survivin has been proposed as an attractive target for new anticancer interventions. Several pre-clinical studies have demonstrated that down-regulation of survivin expression or function, accomplished by means of various strategies, reduced tumor growth potential, increased the apoptotic rate and sensitized tumor cells to chemotherapeutic drugs and radiation in different human tumor models. Moreover, the first survivin inhibitors recently entered clinical trials. Recent studies suggest a possible role for survivin in regulating the function of normal adult cells. However, the expression and function of survivin in normal tissues are still not well characterized and understood. Better knowledge of the role of survivin in tumor versus normal cells will be instrumental for the design of optimal strategies to selectively disrupt survivin in cancer.

Introduction

The anti-apoptotic proteins that counteract signaling through specific apoptosis pathways provide targets for possible drug discovery and new anticancer interventions.

Two major pathways of apoptosis have been identified in mammalian cells. An extrinsic pathway is triggered by the binding of ligands to cell-surface trimeric membrane death receptors and leads to caspase-8 activation (1). An intrinsic pathway involves mitochondria, which respond to pro-apoptotic signals by releasing cytochrome c, which in turn binds and activates the apoptotic protease-activating factor-1, causing assembly of a multiprotein caspase-activating complex (apoptosome) and leading to activation of caspase-9 and initiation of a protease cascade (2). The intrinsic and extrinsic pathways for apoptosis converge on caspase-3, the executor caspase. Some of the targets, such as caspase-3 and caspase-7, are targets of suppression by an endogenous family of anti-apoptotic proteins called inhibitor of apoptosis proteins (IAPs), which also interfere with caspase-9 processing, the upstream initiation of the mitochondrial pathway of apoptosis (3). The human genome encodes eight IAP family members including X-linked inhibitor of apoptosis protein (X-IAP), cIAP1, cIAP2, ML-IAP (Livin; K-IAP), Naip, ILP2 (TS-IAP), Apollon/Brace and survivin (4).

Structure and function of survivin

The human survivin gene spans 14.7 kb on the telomeric position of chromosome 17 and is transcribed from a TATA-less, GC-rich promoter (5) to generate the wild-type transcript and four different splice variant mRNAs (6). Survivin is a 16.5 kD protein of 142 amino acids and is composed of a single baculovirus IAP repeat domain and an extended C-terminal α-helical coiled-coil domain; it does not contain the RING-finger domain found in other IAPs (7).

Growing evidence points to a functional role of survivin in both cell division and apoptosis control (8). Survivin is a chromosomal passenger protein that localizes to kinetochores at metaphase, transfers to the central spindle mid-zone at anaphase and accumulates in mid-bodies at telophase (9). Physical interactions with the inner centromere protein, Aurora B (10,11) and Borealin/Dasra B (12) are required to target the complex to the kinetochore, properly form the bipolar spindle and complete cytokinesis (10–12). Such a function of preservation of genome fidelity and regulation of microtubule dynamics requires a sharp cell-cycle-dependent transcription of the survivin gene during the mitotic phase (13) as well as post-translational modifications of the protein including phosphorylation by the p34^cdc2 (14) and Aurora B (15) kinases and monoubiquitination through Lys48 and Lys63 linkages (16). It has been recently suggested that this pathway is dominant in normal cells and constitutes the primary function of survivin in adult tissues (8,17). However, an up-regulation of survivin in G2/M cell compartments has been observed in various cancer cell lines (18,19).

Other non-cell-cycle-dependent mechanisms driving survivin gene transcription independent of mitosis have been described, which involve tissue patterning circuits (Wnt/β-catenin) (20), cytokine activation signal-transducer-and-activator-of-transcription-3 (21), costimulatory messages (OX-40) (22) and pleiotropic signaling mechanisms [v-akt murine thymoma viral oncogene homolog 1 or protein kinase B (AKT) and nuclear factor-κ B] (23) that are operative during development and generally up-regulated in cancer cells (24) and can explain survivin over-expression in the large majority of human tumors. It has been recently suggested that these non-cell-cycle-dependent pathways are dominant in tumors. This hypothesis also relies on the fact that a transgenic mouse model expressing the green fluorescent protein reporter gene under the control of the minimal survivin promoter demonstrated that expression of survivin in development and tumor formation is largely independent of cell-cycle-dependent transcription of the survivin gene at mitosis (25). The fraction of survivin produced through these non-cell-cycle-dependent mechanisms mediates apoptosis inhibition through intermolecular cooperation with cofactors including the hepatitis B virus X-interacting protein (26), a target of the oncogenic viral hepatitis B virus X protein and X-IAP (27), leading to the formation of complexes that inhibit caspase-9 processing. Moreover, subcellular compartmentalization of survivin in mitochondria seems to play a role in the anti-apoptotic function of the protein. Specifically, the existence of a mitochondrial pool of survivin was recently reported, and it was found that in response to cell death stimulation, mitochondrial survivin is rapidly discharged and released into the cytosol, where it prevents caspase activation and inhibits apoptosis (28). In addition, survivin was not seen in mitochondria in normal tissues, suggesting that mitochondrial survivin is exclusively associated with tumor transformation (28). A very recent study found that survivin has a nuclear export signal and that in cancer cells the anti-apoptotic and mitotic roles of survivin can be separated through mutation of its nuclear export signal, which abrogates the cytoprotective activity of the protein but still allows mitosis to proceed (29).

A physical interaction with the molecular chaperone heat shock protein 90 (Hsp90), which involves the Hsp90 ATPase domain and the survivin baculovirus IAP repeat domain, was shown to be essential for the stability and function of survivin. In fact, targeted antibody-mediated disruption of the survivin–Hsp90 complex in cancer cells resulted in proteasomal degradation of survivin, mitochondrial-dependent apoptosis and mitotic arrest (30). A possible role of survivin...
in the concluding paragraph, it has been recently suggested considering that ectopic expression of survivin in colon cancer cells was able to enhance telomerase activity through the up-regulation of Sp1- and c-Myc-mediated transcription of the human telomerase reverse transcriptase (hTERT) component of the enzyme (31).

Little is known about the functions of alternative splice forms of survivin, which are generally expressed at lower levels than the wild-type survivin. Preliminary data suggest that heterodimerization of wild-type survivin with survivin-ΔEx3 is essential for the inhibition of mitochondrial-dependent apoptosis (32). It has also been demonstrated in exogenous expression assays that survivin-2x splice variant attenuates the anti-apoptotic activity of wild-type survivin (33). Moreover, it was reported that despite their ability to interact with wild-type survivin, alternative splice isoforms such as survivin-ΔEx3 and survivin-2b do not play a role in mitosis since they do not localize with the chromosomal passenger complex in vivo as a consequence of their reduced affinity for the survivin partner Borealin (34). Moreover, these splice variants cannot rescue cell proliferation inhibited by RNA interference (RNAi)-mediated survivin depletion (34).

Survivin expression in normal and tumor tissues

Survivin expression in normal tissues is developmentally regulated and the protein was found to be absent or low in most terminally differentiated tissues (5). However, recent studies tend to attribute a role to survivin in regulating the function of normal adult cells (35) including vascular endothelial cells (36), polymorphonuclear cells (37), T cells (38), erythroid cells (39) and hematopoietic progenitor cells (40). Moreover, survivin expression was reported in adult liver cells (41), gastrointestinal tract mucosa (42) and ovarian granulosa cells (43). However, although survivin is expressed in normal tissues characterized by self-renewal and proliferation, its expression is significantly lower than in transformed cells. In fact, several studies have demonstrated strong survivin expression in most human solid tumor types and hematologic malignancies (44). Expression of survivin has also been detected in a variety of benign and preneoplastic lesions including melanocytic nevi (45,46), polyps of the colon, breast adenomas, Bowen’s disease and hypertrophic actinic keratosis (44), suggesting that re-expression of survivin may occur early during malignant transformation or following a disturbance in the balance between cell proliferation and cell death. The up-regulation of survivin at the transcriptional level in human tumors has been confirmed in genome-wide searches, which indicated survivin as the fourth top “transcriptome” in cancers of various histology (47). Moreover, global deregulation of the survivin gene mediated by oncogenes such as signal-transducer-and-activator-of-transcription-3 (21), E2F (48) and activated H-Ras (49,50) or by loss of tumor suppressors like wild-type p53 (51) or the adenomatous polyposis coli protein (52) seems to be responsible for the enhanced expression of survivin in tumors.

Growing evidence suggests that survivin expression in cancer cells is associated with clinicopathologic variables of aggressive disease and may represent an important prognostic marker for patient outcome. In fact, several studies on different types of solid tumors and hematologic malignancies showed that high levels of the protein were predictive of tumor progression in terms of either disease-free or overall survival (44). In several neoplasms, the association with tumor progression was corroborated in the context of a comprehensive analysis of gene-expression profiling by DNA microarray or polymerase chain reaction-based assay. As it is possible to immunohistochemically distinguish two intracellular pools of survivin, a nuclear and a cytosolic one, the prognostic significance of the protein has been analyzed in some studies as a function of its intracellular localization, and inconsistent and sometimes contrasting results have been obtained regarding the prognostic value of nuclear survivin expression (53). The different prognostic value of survivin may reflect differences in methods to detect its expression and/or a differential expression of survivin splice variants. For instance, expression of the 2b variant, which does not seem to have anti-apoptotic activity, was found to predominate in neuroblastomas with a good prognosis (54).

Survivin as a determinant of treatment resistance

There is evidence that survivin plays an important role in the drug-resistant phenotype of human cancer cells. Giodini et al. (55) first reported that infection of HeLa cells with an adenoviral vector expressing survivin suppressed apoptosis induced by taxol. Successfully, we found that stable transfection of OAW42 and IGROV-1 human ovarian cancer cell lines with survivin cDNA was able to protect the cells from the cytotoxic effects induced by taxol and taxotere, with IC_{50} values for the survivin-transfectant cell populations 4- to 6-fold those of control cells (56). Zhang et al. (57) showed that forced expression of wild-type survivin in human prostate cancer cell lines increased the resistance to taxol in vitro and in vivo. In the clinical setting, when we analyzed the response of 95 patients with advanced ovarian cancer to a taxol-based regimen as a function of survivin expression, we found significantly higher clinical or pathologic response rates in cases with absent or low protein expression than in those expressing high levels of survivin (56).

It has been reported that taxol-induced microtubule stabilization and mitotic arrest increase the expression of survivin, which engenders a cell survival pathway to counteract taxol-induced apoptosis (58). Interestingly, using a taxol-resistant ovarian cancer cell clone, PTX10, with a β-tubulin mutation at the taxol-binding site, Zhou et al. (59) found that taxol treatment failed to induce mitotic arrest and survivin expression. However, the finding that taxol induced an apoptotic response in these cells suggests that mitotic arrest is not strictly required for taxol-induced apoptosis. It is also possible that the mitotic survival pathway is not the only one by which cancer cells counteract taxol-induced apoptosis. In fact, Ling et al. (60) recently reported that induction of survivin by taxol in MCF-7 cells is an early event and is independent of taxol-mediated G2/M arrest, suggesting a role for survivin in taxol resistance not only during mitosis but also outside of the mitotic checkpoint. There is evidence that the mammalian target of rapamycin pathway, which constitutes a sensor network for stress conditions, is involved in resistance to taxol by increasing survivin levels (61). In fact, it has been recently reported that insulin-like growth factor-1-mediated mammalian target of rapamycin activation in prostate cancer cells positively modulated survivin levels by favoring the utilization of a survivin mRNA pool and that mammalian target of rapamycin inhibition with rapamycin, alone or in combination with taxol, abolished survivin increase. Consistent with a critical reduction of the anti-apoptotic threshold maintained by survivin, the taxol plus rapamycin combination was more effective than either treatment in reducing cell viability in the presence of insulin-like growth factor-1 (61).

An increase in survivin expression has also been reported in prostate cancer (62) and thyroid cancer (63) cell lines permanently resistant to cisplatin as well as in colorectal cancer cells resistant to tumor necrosis factor-related apoptosis-inducing ligand (64). Zhang et al. (65) showed that survivin mediates resistance to anti-androgen therapy with flutamide in prostate cancer cells. Specifically, these authors suggested that up-regulation of survivin via insulin-like growth factor-1/AKT signaling during androgen blockade may be one of the mechanisms by which prostate cancer cells develop resistance to anti-androgens. Paik et al. (66) showed that survivin was one of the sixteen genes predictive of recurrence in tamoxifen-treated breast cancer patients.

Regarding the role of survivin in determining the response of human tumor cells to radiation, Asanuma et al. (67) first reported that survivin acts as a constitutive radiosensitivity factor in pancreatic cancer cells. Specifically, in a panel of established cell lines, they found an inverse relationship between survivin mRNA expression and in vitro sensitivity to X-irradiation. Moreover, they demonstrated that survivin mRNA expression was increased by sublethal doses of X-irradiation, which would suggest that the protein also acts as an inducible radiosensitivity factor. Rodel et al. (68) also showed an...
inverse correlation between survivin expression and apoptotic response to irradiation in a panel of colorectal carcinoma cell lines. More recently, in a translational study of 59 rectal cancer patients treated with a combination of radiotherapy and chemotherapy, the same authors reported that increased survivin expression was associated with a significantly increased risk of local tumor recurrence (69). It is worthy of note that survivin can contribute to radiation resistance also by promoting the survival of tumor vascular endothelial cells. In fact, induction of vascular endothelial apoptosis was recently shown to be a major determinant of overall tumor response to radiotherapy (70). Radiation may induce tumor cells to secrete cytokines such as vascular endothelial growth factor, which in turn could inhibit radiation-induced apoptosis of vascular endothelial cells by up-regulating survivin expression, as already demonstrated for drug-induced apoptosis (71).

Survivin-directed cancer therapy

In recent years, considerable efforts have been made to validate survivin as a new target in cancer therapy. In this context, a collection of different approaches to counteract survivin in tumor cells (Figure 1) have been proposed with the dual aim to inhibit tumor growth potential and to enhance tumor cell response to apoptosis-inducing anticancer agents.

Molecular antagonists

Anti-sense oligonucleotides

Grossman et al. (73) first demonstrated that transfection of survivin anti-sense triggered spontaneous apoptosis in the absence of other stimuli in YUSAC2 and LOX human melanoma cell lines. Successively, several studies dealing with the use of survivin anti-sense oligonucleotides, delivered to the cells as chemically synthesized molecules or through the use of expression vectors, consistently showed that specific inhibition of survivin mRNA and protein could reduce cell proliferation and induce caspase-dependent apoptosis in established cell lines of different tumor origins including lung, bladder, head and neck and thyroid cancers, sarcomas and lymphomas (74–78). Moreover, down-regulation of survivin was shown to sensitize human tumor cells to cytotoxic drugs such as etoposide and cisplatin (75), as well as ionizing radiation (79). Anti-sense-mediated survivin knock-down also caused inhibition of tumor growth in xenograft models (74,78) and sensitized lung cancer xenografts to radiotherapy (74). Kanwar et al. (80) demonstrated that survivin inhibition resulted in increased sensitivity to immunotherapy in murine EL-4 thymic lymphoma. Specifically, tumors injected with plasmids encoding survivin anti-sense were significantly inhibited in their growth. Such growth delay was further enhanced by concomitant injection of the T-cell costimulator B7-1.

Supported by a favorable safety profile, the first anti-sense oligonucleotide, LY2181308 (ISIS 23722; Lilly and Co., Indianapolis, IN and ISIS Pharmaceuticals, Carlsbad, CA, US), already entered the clinic and is currently undergoing Phase I trials in patients with advanced cancers.

Hammerhead ribozymes

As an alternative anti-sense strategy for survivin inhibition, we developed in our laboratory two hammerhead ribozymes targeting the 3’ end of the CUA110 (RZ7) and the GUC294 (RZ1) triplets in the survivin mRNA and transfected them into the JR8 human melanoma cell line over-expressing survivin. Stably transfected clones proven to endogenously express the active ribozyme RZ1 or RZ7 were characterized by a markedly lower survivin protein level than JR8 parental cells, and showed an increased caspase-9-dependent apoptotic response to treatment with the cytotoxic agents cisplatin (81) and topotecan (82) as well as with γ-irradiation (83). Moreover, an increased antitumor activity of oral topotecan was observed in ribozyme-expressing JR8 cells grown as xenograft tumors in athymic nude mice (82). In addition, we constructed a Moloney-based retroviral vector expressing the RZ7 ribozyme, encoded as a chimeric RNA within adenoviral VA1 RNA. Polyclonal cell populations, obtained by infection with the retroviral vector, of two androgen-independent human prostate cancer cell lines (DU145 and PC-3) were characterized by a significant reduction of survivin expression; the cells became polyploid, underwent caspase-9-dependent apoptosis and showed an altered pattern of gene expression, as detected by oligonucleotide array analysis. Survivin inhibition also enhanced the susceptibility of these cells to cisplatin-induced apoptosis and prevented tumor formation when cells were xenografted into athymic nude mice (84). Choi et al. (85) also showed that two hammerhead ribozymes, able to cleave the human survivin mRNA at nucleotide position +279 and +28 and cloned into a replication-deficient adenoviral vector, increased the apoptotic response to etoposide in transduced MCF-7 breast cancer cells.

Small interfering RNAs

The discovery that synthetic 21–23 nucleotide RNA duplexes can trigger an RNAi response in mammalian cells and induce strong inhibition of specific gene expression has opened the door to the therapeutic use of small interfering RNAs (siRNAs) (86). Carvalho et al. (87) first used RNAi to specifically repress survivin in HeLa cells. These authors showed that survivin was no longer detectable in cultures 60 h after transfection with specific siRNAs and that survivin-depleted cells were delayed in mitosis and accumulated in prometaphase with misaligned chromosomes. Several studies dealing with the use of chemically synthesized siRNAs or plasmid/viral vectors encoding short hairpin RNAs showed that RNAi-mediated survivin knock-down was able to reduce tumor cell proliferative potential and induce caspase-dependent apoptosis in a variety of human tumor cell models (17,88–93), as well as to decrease the formation of new
tumors and the growth of already established lesions in nude mice (93,94). Survivin down-regulation also induced an enhanced apoptotic response of tumor cells of different histologic origin to several stimuli, including treatment with vincristine (93), doxorubicin (94,95), 17-allylamino-17-demethoxygeldanamycin (89), tumor necrosis factor-alpha (94) and APO2 ligand/tumor necrosis factor-related apoptosis-inducing ligand (90), and caused radiosensitization of a sarcoma cell line expressing wild-type p53 (96). Finally, Coma et al. (97) demonstrated that transfection of endothelial cells with survivin-specific siRNAs induced a marked increase in the rate of apoptosis, a dose-dependent inhibition of their migration on vitronectin, and a decrease in capillary formation.

Gene therapy

Two main gene therapy approaches targeting survivin have been successfully developed. One is based on the use of plasmids or viral vectors to deliver dominant-negative survivin mutants to tumor cells. Grossman et al. (73) first demonstrated that transfection of YUSAC2 and LOX melanoma cell lines with a mutant carrying a cysteine 84 → alanine (Cys84Ala) mutation in the survivin baculovirus IAP repeat domain increased the apoptotic index and enhanced the sub-G1 apoptotic cell fraction in both tumor models. More recently, Tu et al. (98) showed that adenovirus-mediated transfer of the survivin mutant Cys84Ala induced apoptosis and mitotic catastrophe in colon cancer cells, inhibited angiogenesis and tumor growth in a colon cancer xenograft model in vivo and strongly enhanced the antitumor activity of 5-fluorouracil. By using a phosphorylation-defective survivin threonine 34 → alanine (Thr34 → Ala) mutant, Grossman et al. (99) demonstrated the induction of spontaneous apoptosis in different melanoma cell lines as well as an increased apoptotic response following cisplatin treatment. Conditional expression of Thr34 → Ala in these cells prevented tumor formation upon subcutaneous injection in 13 of 15 CB.17 severely combined immunodeficient mice. Such treatment also caused a significant reduction (60–70%) in the growth rate of already established tumors and enhancement of apoptosis (99). Mesri et al. (100) investigated the effect of a replication-deficient adenovirus encoding the survivin Thr34 → Ala dominant-negative mutant (pAd-T34A) in human tumor cells of various origin in vitro and in vivo. They showed that infection with pAd-T34A caused enhanced spontaneous and taxol-induced apoptosis in cell lines of different tumor origin while not affecting the growth of proliferating normal human cells not expressing survivin. In vivo experiments on the MCF-7 human breast cancer xenograft model demonstrated that pAd-T34A was able to suppress de novo tumor formation, inhibit the growth of already established tumors by ~40% and reduce intra-peritoneal tumor dissemination. Moreover, tumors infected with pAd-T34A showed massive apoptosis and loss of proliferating cells. Very recently, using a recombinant fusion protein containing the TAT transduction domain and the Thr34 alanine (Thr34Ala) mutant, Grossman et al. (101) observed an induction of caspase-dependent apoptosis in melanoma cells. Moreover, repeated intra-peritoneal injection of TAT-Surv-T34A resulted in a marked reduction of the growth and mass of established subcutaneous tumors in mice.

The second gene therapy approach involves the use of the survivin gene promoter to drive the expression of cytotoxic genes in tumor cells (102). It was shown that when coupled to the pro-apoptotic protein Bik, administration of the suidical construct as a DNA–liposome formulation suppressed the growth of human lung cancer in vitro and in vivo (103). Van Houdt et al. (104) showed recently that survivin promoter-based conditionally replicative adenoviruses (CRAds), composed of survivin promoter-regulated E1 gene expression and an RGD-4C capsid modification, efficiently replicated within and killed a variety of established glioma cell lines but were inactive in a normal liver culture. Moreover, survivin promoter-based CRAds significantly inhibited the growth of glioma xenografts in vivo.

Small molecule antagonists

Cyclin-dependent kinase inhibitors

In the context of a strategy focused on pharmacologic inhibition of mitotic phosphorylation of survivin on Thr34 to accelerate protein destruction and counteract its function (58), cyclin-dependent kinase (CDK) inhibitors such as flavopiridol or the more p34S/cdc2-specific inhibitor, purvalanol A, were tested in tumor cells arrested at mitosis with taxol, which induces hyperphosphorylation of survivin on Thr34 (56). Sequential administration of CDK inhibitors resulted in escape from the mitotic block imposed by taxol, marked activation of mitochondrial-dependent apoptosis and anticaner activity in vivo (58). More recently, inhibition of survivin phosphorylation and expression was also suggested to be the potential mechanism by which the novel CDK inhibitor NU6140 (4-(6-cyclohexylmethoxy-9H-purin-2-ylamino)-N,N-diethyl-benzamide) potentiates taxol-induced apoptosis in HeLa cells (105).

Hsp90 inhibitors

A structure-based rational screening for antagonists of the survivin–Hsp90 complex identified a cell-permeable peptidomimetic derived from the survivin sequence Lys78–Leu87, shepherdin (106). In addition to counteracting survivin–Hsp90 interaction, the molecule inhibited Hsp90 chaperone function by competing with ATP binding. Specifically, shepherdin was able to destabilize several Hsp90 client proteins (including Akt, CDK6 and telomerase) and to induce cell death via apoptotic and non-apoptotic mechanisms in cell lines of different tumor types. The cytotoxicity of shepherdin was seen to be independent of the proliferative activity of the cell model, concomitant over-expression of other cytoprotective factors such as Bcl-2 and p53 status. Moreover, shepherdin appeared to be selective in its anti-tumor activity since it did not affect viability of normal cells or tissues, including hematopoietic progenitors. Systemic administration of shepherdin in vivo was safe and well tolerated and inhibited the growth of already established prostate and breast cancers without inducing systemic or organ toxicity. Moreover, immunohistochemical analysis of Hsp90 client proteins carried out in tumor samples at the end of shepherdin treatment showed a nearly complete loss of Akt levels and severely attenuated survivin expression (106). The clinical development of shepherdin with respect to its optimal formulation and pharmacokinetic profile is currently under way through the National Cancer Institute Rapid Access to Intervention Development program.

Since the shepherdin residues between Lys78 and Gly83 were shown to be essential for Hsp90 binding, a new five-residue peptide containing the Lys78–Gly83 sequence (called shepherdin[79–83]) was successively synthesized (107). The oligopeptide inhibited the formation of the survivin–Hsp90 complex and competed with ATP binding to Hsp90. Cell-permeable shepherdin[79–83] induced rapid killing in different types of human acute myeloid leukemia cell lines and in patient-derived acute myeloid leukemia peripheral blasts but not in normal mononuclear cells. Moreover, when systemically delivered, shepherdin[79–83] abolished the growth of acute myeloid leukemia xenograft tumors without systemic or organ toxicity (107). We have recently used shepherdin as a scaffold to rationally identify low-molecular weight compounds that may act as structurally novel Hsp90 inhibitors. Through a combined structure- and dynamics-based computational design strategy, the non-peptidic small molecule 5-aminoimidazol-4-carboxamide-1-beta-d-ribofuranoside (AICAR) was selected to bind the Hsp90 N-terminal domain, mimicking the chemical and conformational properties of shepherdin. AICAR was shown to destabilize several Hsp90 client proteins in vivo, including survivin, and to exhibit anti-proliferative and pro-apoptotic activities in multiple tumor cell lines while not affecting proliferation of normal human fibroblasts (108).

Other small molecules

Additional small molecules that target survivin have been developed and some of them are currently being evaluated in clinical trials. One
of those that directly affect survivin expression, tetra-O-methyl nor-dihydroguaiaaretic acid (M(4)N), was shown to suppress Sp1-dependent survivin gene transcription and activate the mitochondrial apoptotic pathway in transformed cells (109). Moreover, M(4)N suppressed the growth of human xenograft tumors following systemic treatment (110). However, considering that the compound is a global transcription inhibitor, both survivin-dependent and survivin-independent pathways are thought to be responsible for drug-induced cell death in tumors (111).

YM155 is a small molecule that selectively inhibits survivin gene transcription and protein expression in several tumor cell lines. It showed marked anti-proliferative activity (in the nanomolar range) in a broad spectrum of human tumor cell lines and induced tumor regression in lymphoma, prostate cancer and non-small cell lung cancer xenografts (112). Moreover, preliminary evidence of the clinical activity of the compound was derived from a phase I open-label study in which 41 patients with different tumor types were treated with 7-day continuous intravenous infusion of YM155 and objective responses were observed in three patients with non-Hodgkin’s lymphoma (112). The compound is currently being evaluated in a phase II study for patients with stage III and stage IV melanoma.

Conclusions

Results from studies exploiting different strategies to interfere with survivin expression and function provided direct and convincing evidence that targeting the survivin network inhibits tumor growth potential in vitro and in vivo and increases spontaneous and treatment-induced apoptosis of cancer cells, thus indicating survivin as a promising molecular target for cancer therapy. The applicability of survivin-targeted strategies for the clinical treatment of human tumors is currently under investigation as the first survivin inhibitors recently entered phase I–II clinical trials. Although the results of these trials are not yet available, survivin inhibitors may represent a novel type of targeted drugs inasmuch as they specifically interfere with defined molecular pathways of tumor cell maintenance and at the same time are applicable to different tumor types independent of their genetic makeup.

Although the anti-survivin therapies developed thus far have not shown any major systemic toxicity in in vivo experimental models and appear to be extremely promising, the possibility that survivin disruption could affect normal cell function, mainly the hematopoietic and immune systems, cannot be excluded. In this context, a better understanding of the effects exerted by survivin on normal versus malignant cells will be important in identifying strategies that maximally disrupt survivin in cancer cells while minimally affecting normal tissues. Moreover, in accord with the more recent view suggesting that the two main functions of survivin, i.e. spindle monitoring at mitosis and the ability to counteract apoptosis mainly through caspase inhibition, are differentially exploited in normal and tumor cells, the identification of points at which these functions can be separated could open new possibilities for strategies aimed at eliminating cancer cells while preserving normal cell viability.

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Targeting survivin in cancer therapy


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