

Targeted therapy by disabling *crossroad* signaling networks: the survivin paradigm

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Abstract

Embedded in the concept of targeted cancer therapy is the expectation that disabling a single oncogenic pathway will eliminate the tumor cells and leave the normal tissues unscathed. Although validated by clinical responses in certain malignancies, challenges exist to generalize this approach to most tumors, as multiple genetic lesions, chromosomal instability, insensitivity of the cancer stem cell compartment, and emergence of drug resistance complicate the identification and therapeutic exploitation of a single, driving oncogenic pathway. Instead, broader therapeutic prospects may be offered by targeting *crossroad* signaling networks that are selectively exploited in cancer and oversee multiple aspects of tumor cell maintenance. One such pathway is centered on *survivin*, a cancer gene that intersects cell proliferation, cell survival, and the cellular stress response. Several clinical trials targeting survivin with a collection of approaches from immunotherapy to small-molecule antagonists are currently under way. By simultaneously disabling multiple signaling circuitries, targeting survivin may provide a novel perspective in rational cancer therapy selective for specific cancer mechanisms but broadly applicable to disparate tumors regardless of their genetic makeup. [Mol Cancer Ther 2006;5(3):478–82]

Biology of *Survivin*: A Cancer Gene Deeply Wired with Fundamental Signaling Circuitries

At 16.5 kDa, *survivin* is the smallest member of the inhibitor of apoptosis gene family (1). These molecules contain one to three zinc finger folds and act as evolutionary conserved suppressors of caspases, the effector enzymes of apoptosis

(1). Differently from other inhibitor of apoptosis, survivin forms a stable homodimer in solution and is essential in that deletion of the *survivin* gene in mice causes early embryonic lethality and immediate loss of tissue/organ viability.

Two general features make survivin unique: its wiring with multiple signaling circuitries (Fig. 1) and its differential expression in cancer compared with normal tissues. Preferentially expressed at mitosis in a cell cycle-dependent manner and physically associated with the mitotic apparatus, survivin is essential for proper completion of various stages of cell division, from centrosomal functions to proper kinetochore attachment to spindle formation (Fig. 1), potentially via regulation of microtubule dynamics/stability (2). Survivin is also a genuine apoptosis inhibitor, counteracting cell death by interfering with caspase-9 processing (3), the upstream initiation of the intrinsic (mitochondrial) pathway of apoptosis. Lastly, these combined properties are exploited during the cellular stress response, in which binding of survivin to the molecular chaperone Hsp90 (4) helps cells cope with unfavorable environments, preserving cell proliferation and cell viability (Fig. 1).

The second broad feature of *survivin* is its role as a cancer gene, providing for the top 4th “transcriptome” expressed in transformed cells compared with normal tissues (2). This reflects an “oncofetal” pattern of expression, as survivin is abundantly and ubiquitously present in development, undetectable in most adult tissues, and prominently reexpressed in virtually every human cancer. A global deregulation of the *survivin* gene mediated by oncogenes, including STAT3 (5), E2F (6), or mutated Ras (7), or by loss of tumor suppressors, like p53 (8) or the adenomatous polyposis coli protein (9), accounts for the selective expression of survivin in cancer. This carries prognostic and predictive implications and is consistently associated by molecular profiling with advanced disease, high grade, abbreviated survival, resistance to therapy, and accelerated recurrences (2). Clinical exploitation of *survivin* for cancer molecular diagnostic is under way, with its inclusion as 1 of 16 genes predictive of recurrences in breast cancer (10) and as a urine biomarker in bladder cancer (11).

Survivin-Directed Cancer Therapy

Because of its role as a cancer gene intersecting multiple cellular networks (Fig. 1), *survivin* has been vigorously pursued as a cancer drug target. Compared with other

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apoptosis-based cancer therapies (12), survivin provides several advantages. First, disabling survivin is expected to compromise multiple signaling networks required for tumor cell maintenance. This is important because targeted therapies aiming at a single oncogenic pathway quickly elicit resistance, frequently through mutations of drug contact site(s) (13). Second, survivin may be a uniquely flexible target, suitable for molecular antagonists, vaccination strategies, small-molecule inhibitors, and gene therapy. Third, survivin expression is regulated by developmental signaling pathways operative in stem cells (i.e., Wnt) and it is possible that survivin antagonists may affect cancer stem cells (14), a compartment largely untouched by cytotoxics or targeted agents (15). Fourth, survivin is important in ancillary aspects of tumor formation/progression, especially angiogenesis (16, 17), and survivin inhibitors have been shown to act on both the transformed population and endothelial cells in the tumor mass. Fifth, although survivin expression has been shown in cytokine-stimulated hematopoietic progenitors and potentially in activated T cells, targeting this pathway did not affect normal cells or tissues in preclinical or phase I studies (see

below), suggesting a favorable toxicity profile of survivin-based therapeutics. Below is a brief discussion of the portfolio of strategies to target survivin for novel cancer therapies (Table 1).

Molecular Antagonists

The first molecular antagonist of survivin was a phosphorothioate-modified antisense oligonucleotide reported in 1999 (18). This reagent along with similar others later developed suppressed survivin mRNA and protein expression and produced strong anticancer activity with inhibition of tumor cell proliferation, spontaneous apoptosis, enhancement of cytotoxics or ionizing radiation, and inhibition of tumor growth in xenograft models (19). Supported by a favorable safety profile, the original survivin antisense oligonucleotide has now completed a phase I trial in patients with advanced cancers and a phase II trial has been announced. A parallel strategy to suppress survivin levels in tumor cells involved RNA interference (20, 21) or hammerhead ribozymes (22). These reagents produced a phenotype similar to antisense and passed proof-of-concept with inhibition of tumor growth in xenograft models (20, 22, 23).

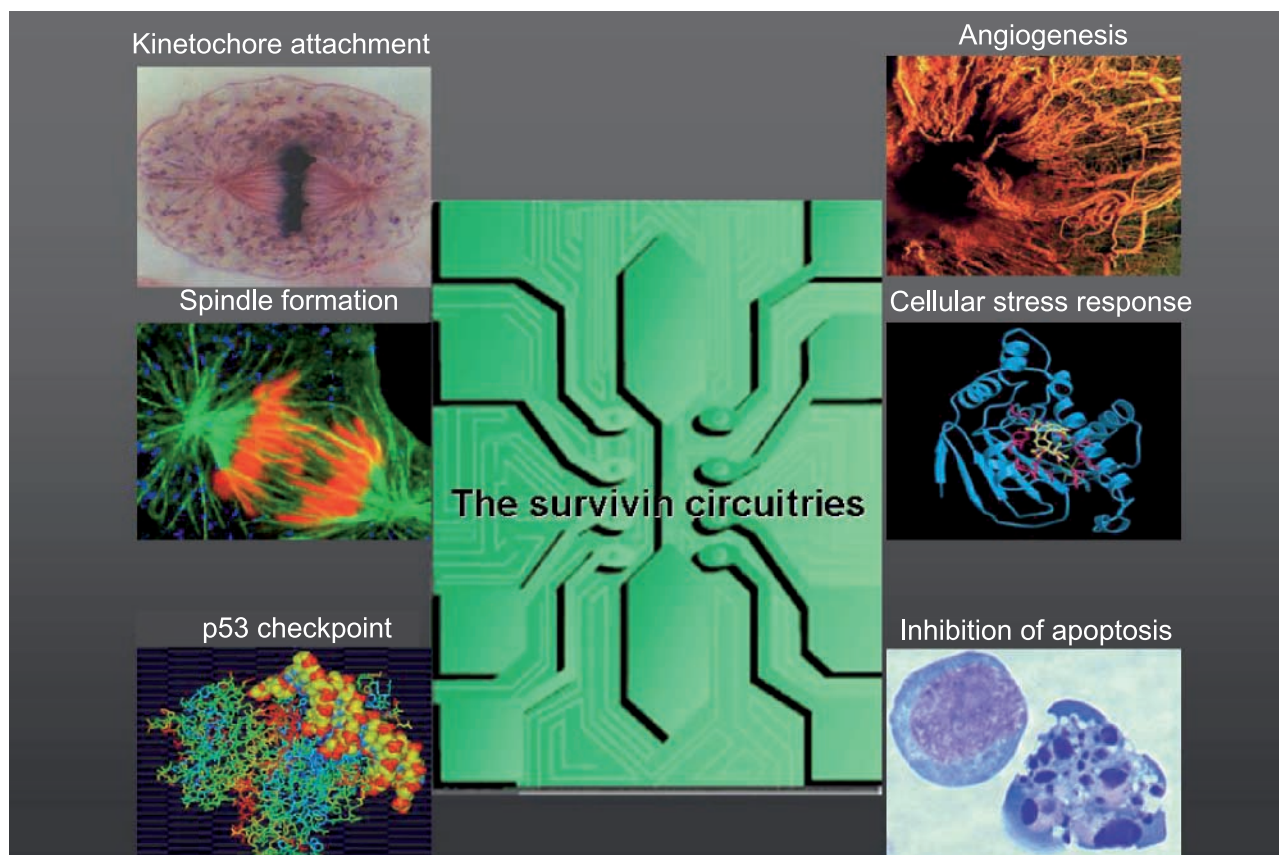


Figure 1. Molecular circuitries of survivin. Survivin intersects multiple signaling networks implicated in inhibition of apoptosis primarily by targeting the intrinsic (i.e., mitochondrial) pathway of cell death, modulation of p53 checkpoint(s), and control of spindle formation and proper kinetochore attachment during cell division. Survivin has also critical functions in preserving endothelial cell viability during the proliferative and remodeling phases of angiogenesis and participates in the cellular stress response by associating with the molecular chaperone Hsp90.

Table 1. Current therapeutic strategies for targeting the survivin pathway in cancer

Therapeutic approach	Compounds	Preclinical	Clinical development	Refs.
Antisense	LY2181308 (ISIS/Eli Lilly)	Completed	Phase II trial	(18, 19)
Molecular antagonists	Ribozyme RNA interference	Ongoing	Not yet started	(20–23)
Gene therapy	Dominant interfering mutants (Cys ⁸⁴ A; T34A)	Ongoing	Not yet started	(36–40)
Gene therapy	Survivin-directed transcriptional delivery of cytotoxic gene(s)	Ongoing	Planned	(41–43)
Small-molecule antagonist	Tetra- <i>O</i> -methyl nordihydroguaiaretic acid	Completed	Phase I trial	(44)
Small-molecule antagonists	STAT3 Cdk1 (flavopiridol) T-cell factor Hsp90 (17-allyl-amino-geldanamycin) ErbB2 (lapatinib)	Completed	Phase I-II trials	(45–49)
Immunotherapy	Autologous CTL pulsed with survivin-primed dendritic cells	Completed	Phase I and II trials	(26–28, 33–33)
Immunotherapy	Oral DNA vaccine	Ongoing	Planned	(32)
Shepherdin	Combined survivin/Hsp90 antagonist	Ongoing	NCI-RAID	(51)

Cancer Vaccine/Immunotherapy

Because of its differential expression in cancer, it was hypothesized that cancer patients may recognize survivin as a nonself protein and mount an immune response to it (24). This has been validated in the clinic, and sera from cancer patients contain antibodies (25) and cytolytic T cells against survivin (26). This recognition has been mapped in detail (27, 28), and dendritic cells pulsed with survivin peptides, or expressing survivin, elicit cytolytic T cells, which exhibit MHC-restricted anticancer activity *in vitro* (29, 30) and in preclinical models (31). When used as an oral DNA vaccine, the survivin-directed immune response affected both tumor cells and tumor-associated angiogenesis, eradicating pulmonary metastases without toxicity in preclinical studies (32). Survivin-directed immunotherapy has been quickly moved to the clinic, and several phase I trials with administration of survivin peptides or survivin-directed autologous CTL generated *ex vivo* have been recently completed (33–35). Survivin-based vaccination was found to be safe, devoid of significant side effects, and frequently associated with antigen-specific immunologic responses (33, 34). Some of these protocols have now been moved into larger phase II trials.

Gene Therapy

Several gene therapy approaches targeting survivin have been developed and passed proof-of-principle in preclinical studies. One approach included plasmid or adenoviral delivery of survivin “dominant-negative” mutants, particularly a survivin Thr³⁴Ala variant that abolishes a phosphorylation site for the main mitotic kinase p34cdc2. This mutant is unstable *in vivo* (36), and its dimerization with endogenous survivin may result in accelerated degradation of the complex and sudden loss of survivin levels. In turn, this causes inhibition of cell proliferation, induction of apoptosis, suppression of tumor growth, and enhancement of cytotoxics or immunotherapy in preclinical models (37–40). A second approach involved the use of the *survivin*

gene to express a “payload” cytotoxic gene in tumor cells. This “suicidal” strategy (41) relies on the fact that the *survivin* gene has virtually no transcriptional expression in normal tissues, including liver (42), as opposed to a 200-fold increased activity in tumor cells *in vivo* (43). When coupled to a proapoptotic protein, administration of the suicidal construct as a DNA-liposome formulation resulted in complete tumor eradication in xenograft models (43), thus offering good prospects for further clinical development.

Small-Molecule Antagonists

Small molecules that either directly or indirectly target survivin have been developed, and several clinical trials using these reagents are under way. At least two phase I trials with small-molecule inhibitors that *directly* target survivin are approaching completion. One of these molecules, tetra-*O*-methyl nordihydroguaiaretic acid was shown to function by suppressing Sp1-dependent *survivin* gene expression, resulting in concomitant activation of mitochondrial apoptosis in tumor cells (44). Several small-molecule antagonists currently in the clinic *indirectly* affect survivin levels. These include cyclin-dependent kinase inhibitors (45), antagonists of STAT3 (46), T-cell factor (47), Hsp90 (48), and ErbB2 (lapatinib; ref. 49). These compounds reduce survivin levels by different mechanisms, including accelerated protein destruction via inhibition of Cdk1 phosphorylation (50), suppression of *survivin* gene transcription (T-cell factor and STAT3), protein mislocalization/misfolding (geldanamycin/17-allyl-amino-geldanamycin), or interference with critical intracellular signals (lapatinib). The acute loss of survivin levels under these conditions may contribute to anticancer activity and provide an easily accessible biomarker for target validation in human trials.

Shepherdin

Of all the strategies mentioned above, the targeted disruption of the survivin-Hsp90 complex (4) likely carries the broadest possible effect for tumor cells given the pivotal roles of both molecules in cell proliferation, cell survival,

and cellular adaptation. Clearly, both survivin and Hsp90 are proven “druggable” targets, and several phase I trials with the ansamycin small-molecule Hsp90 antagonist, 17-allyl-amino-geldanamycin (48), have been completed. A recent screening for antagonists of the survivin-Hsp90 complex identified Shepherdin, a cell-permeable peptidomimetic derived from the survivin sequence Lys⁷⁹-Leu⁸⁷ (51). Shepherdin physically inhibited the survivin-Hsp90 interaction but also acted as a global antagonist of Hsp90 chaperone function by competing with ATP binding. Altogether, this resulted in sudden loss of mitochondrial integrity, degradation of multiple Hsp90 client proteins, and massive activation of caspase-dependent and caspase-independent cell death. Shepherdin is unusual for its very rapid and very potent anticancer activity, which seems unaffected by the proliferative condition of tumor cells, overexpression of survival proteins, or p53 status (51). In preclinical studies, Shepherdin ablated tumor growth as single agent in xenograft models, with no toxicity for normal tissues, including purified CD34⁺ progenitor cells (51). It is unclear why Shepherdin is so much more potent than 17-allyl-amino-geldanamycin as an anticancer agent, but this may reflect its expansive and unique engagement of the Hsp90 ATP pocket (51), potentially disabling novel chaperone functions that are not adequately targeted by 17-allyl-amino-geldanamycin. The clinical development of Shepherdin is actively under way through the National Cancer Institute (NCI) Rapid Access to Intervention Development (RAID) with respect to optimal formulation and pharmacokinetic profile.

Future Prospects

Despite its recent discovery in 1997, survivin has provided unique opportunities for basic and translational clinical oncology. In a relatively short period, multiple strategies targeting the survivin network have quickly passed proof-of-principle, and many have entered clinical testing in humans. Although the results of these trials are awaited, the generation of survivin antagonists may provide a novel concept in “targeted” cancer therapy: specific enough to disable defined molecular pathways of tumor cell maintenance but also broad enough to be applicable to disparate tumor types regardless of their genetic heterogeneity.

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