Review

Survivin, a cancer target with an emerging role in normal adult tissues

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Abstract

Survivin, an inhibitor of apoptosis protein, is highly expressed in most cancers and associated with chemotherapy resistance, increased tumor recurrence, and shorter patient survival, making antisurvivin therapy an attractive cancer treatment strategy. However, growing evidence indicates that survivin is expressed in normal adult cells, particularly primitive hematopoietic cells, T lymphocytes, polymorphonuclear neutrophils, and vascular endothelial cells, and may regulate their proliferation or survival. In preclinical animal models, targeted antisurvivin therapies show efficacy without overt toxicity. However, consequences of prolonged survivin disruption in normal cells, particularly those associated with continuous renewal, have not been clearly determined. Understanding the role of survivin in normal versus malignant cells will be important in identifying strategies that maximally disrupt survivin in cancer cells with minimal effect on normal tissues. In this review, we summarize the prognostic relevance of survivin in cancer that justifies the pursuit of antisurvivin therapies and discuss differences in survivin expression between normal and cancer cells. We subsequently review expression of survivin in normal adult tissues and evaluate preclinical antisurvivin therapies reported to date in light of emerging roles for survivin in normal physiology, particularly hematopoiesis, angiogenesis, and immune function. [Mol Cancer Ther 2006;5(5):1087–98]

Introduction

The inhibitor of apoptosis protein survivin regulates apoptosis and cell cycle. Survivin expression has been extensively evaluated in cancer (1); however, its expression and function in normal tissues are not well defined. Survivin has been shown to increase tumor resistance to various apoptotic stimuli, primarily through caspase-dependent mechanisms, although it can also block apoptosis in a caspase-independent fashion. Conversely, antagonizing survivin in tumor cells induces apoptosis (1–4). Survivin disruption in HeLa cells induces aberrant mitosis and polyploidy (5) and homozygous survivin deletion in mice results in early embryonic death from disrupted microtubule formation and polyploidy (6), showing a role for survivin in cell division. Although survivin expression is associated with cell cycle progression and regulation of mitosis in most transformed cell systems investigated (1–3, 7), it also regulates G_1-S transition in T lymphocytes (8, 9), normal hematopoietic progenitor cells (10, 11), hepatoma cells (12, 13), and breast cancer cells (14).

Survivin expression in normal tissue is developmentally regulated and has been reported to be low in most terminally differentiated tissues. The aberrant high expression of survivin in cancer cells, with little expression in most normal tissues, makes survivin an attractive anticancer target. However, expression and evidence of potential function for survivin in normal tissues is accumulating, suggesting that survivin expression is not cancer specific. Several antisurvivin preclinical trials in solid tumor models show that disrupting survivin can reduce tumor growth (15–24). However, recent studies have defined a role for survivin in regulating function in normal adult cells, particularly vascular endothelial cells (16, 25), polymorphonuclear cells (26), T cells (9, 27), erythroid cells (28), and hematopoietic progenitor cells (10, 11, 28, 29), suggesting that survivin disruption could have adverse consequences on these cells. In this review, we will summarize the expression and prognostic value of survivin in cancers and its expression and function in normal adult tissues. Understanding the expression, function, and regulation of survivin in normal versus cancer cells will be critical to the design of optimal strategies to selectively eradicate cancer cells without causing adverse effects in normal tissues.

Survivin Is Expressed in Most Cancers and Has Prognostic Value

Strong survivin expression is observed in the vast majority of cancers (Table 1; also reviewed in ref. 1). These include esophageal, lung, ovarian, central nervous system, breast, colorectal, bladder, gastric, prostate, pancreatic,

Table 1. Survivin and cancer

Cancer type (no. patients)	Method*	Survivin		Correlation with elevated survivin		Reference
		Form	Site[†]	Clinicopathologic variables	Survival[‡]	
Esophageal						
(51)	RT-PCR	WT	N	Nodal status	↓	(107)
(57)	Q-RT-PCR	WT	N	NC[§]	↓	(108)
(84)	IHC	WT	N	Node metastasis	↓	(109)
Non–small-cell lung						
(144; resected)	IHC	WT	N	NC	↓	(51)
(48; resected)	IHC	WT	N	Stage (N)	↓	(110)
(83; resected)	RT-PCR	WT	N	NC	↓	(111)
(102; resected)	IHC	WT		NC	↓	(112)
(53; advanced)	IHC	WT	N	NC	↑	(48)
(83; early, resected)	IHC	WT	N; C	Stage (C)	NC	(113)
Ovarian						
(110)	IHC	WT		NC	NC	(114)
(32)	IHC	WT	N	Stage	↓	(52)
(103)	IHC	WT	N	Residual disease	↓	(35)
(49)	IHC	WT	N	Grade; histology	NC	(115)
(124)	IHC	WT		NC; resistance to taxanes	NC	(41)
Central nervous system						
(59; medulloblastoma)	IHC	WT	N	Aggressiveness	↓	(116)
(92; gliobastoma)	WB	WT		Trend-histology; treatment resistance	↓	(117)
(43; astrocyte tumors)	RT-PCR	WT; ΔEx3; 2B	N	Grade	↓	(118)
Breast						
(293; untreated)	IHC	WT	N	NC	↑	(45)
(106; untreated)	RT-PCR	WT; ΔEx3; 2B	N	NC	NC	(46)
(275; untreated)	Q-RT-PCR	WT	N	Grade; type; ER/PR−	↓	(44)
(167; untreated)	IHC	WT	C	NC	↓ (trend)	(40)
Colorectal						
(144)	RT-PCR	WT	N	NC	↓	(102)
(139)	IHC	WT		NC	↓	(119)
(171)	IHC	WT	C	NC	↓	(39)
(49)	IHC	WT	C	NC	↓	(120)
(96)	IHC	WT		Histology	NC	(121)
Melanoma						
(36; sentinel nodes)	RT-PCR	WT	N		↓	(122)
Gastric						
(133)	IHC	WT	N		↑	(47)
Sarcoma						
(89)	RT-PCR	WT	N		↓	(123)
(63)	ELISA; WB	WT		Grade; aggressiveness	↓	(124)
(94)	Q-RT-PCR	WT; ΔEx3	N		↓	(125)
Osteosarcoma						
(40)	IHC	WT	N; C	Tumor size (N)	↑ (N); NC (C)	(49)
Pancreatic						
(52)	IHC	WT	C	NC	↓	(53)
(52)	IHC	WT	C	NC	NC	(126)
Oral; laryngeal						
(110; oral squamous)	IHC; WB	WT	C	NC	↓	(54)
(68; laryngeal squamous)	IHC	WT	N	Site	↓	(55)
Cervical						
(17; squamous)	IHC	WT	N; C	NC	NC	(127)

Abbreviations: ALL, acute lymphoblastic leukemia; FAB, French-American-British classification; WT, wild type.
*IHC, immunohistochemistry; WB, Western blot; RT-PCR, reverse transcriptase PCR; Q-RT-PCR, quantitative RT-PCR; RPA, RNA protection assay.
[†]Nuclear (N) versus cytoplasmic (C) staining.
[‡]Shorter (↓) or prolonged (↑) disease-free (DF) or overall (OS) survival.
[§]NC, no statistical correlation.

(*Continued on the following page*)

Table 1. Survivin and cancer (Cont'd)

Cancer type (no. patients)	Method*	Survivin		Correlation with elevated survivin		Reference
		Form	Site[†]	Clinicopathologic variables	Survival[‡]	
(53; squamous)	IHC	WT	N	Trend-stage	NC	(128)
Endometrial						
(31)	IHC	WT	N	Grade; invasiveness	↓	(36)
Hepatocellular						
(72)	IHC	WT	N	Grade; invasiveness	↓ DF; NC in OS	(37)
(40)	Q-RT-PCR	WT	N	Grade; stage	↓	(38)
(51)	RT-PCR; WB	WT	N	NC	↓	(129)
Hematologic						
AML (45; pediatric)	WB; FC	WT		NC	↓	(130)
AML (116; adult)	WB	WT		NC	NC	(131)
AML (125)	IHC	WT	C	Low WBC; FAB M2 subtype	↓ (trend)	(132)
AML (31; adult)	RT-PCR	WT	N		↓	(33)
ALL (16)	RT-PCR	WT	N		NC	
Large-cell lymphoma	IHC	WT	C		↓	(133)
B-cell lymphoma	IHC	WT			↓	(134)

laryngeal, uterine, hepatocellular, and renal cancers, as well as melanoma and soft tissue sarcomas. Survivin is also highly expressed in patients with hematologic malignancies (reviewed in ref. 30), including lymphomas, acute leukemias, and myelodysplastic syndromes, which progress to overt leukemia. Survivin overexpression is not observed in patients with chronic leukemias, including B-cell chronic lymphocytic leukemia (31), chronic myelomonocytic leukemia (32), and chronic myelogenous leukemia in chronic phase (33, 34). Survivin expression was high in Philadelphia-positive chronic myelogenous leukemia patients in blast crisis (34), suggesting that up-regulation of survivin expression may be involved in evolution of chronic myelogenous leukemia and that survivin expression and hematopoietic cell differentiation may be related (33, 34).

In cancer cells, elevated survivin is commonly associated with enhanced proliferative index (35–38), reduced levels of apoptosis (39, 40), resistance to chemotherapy (41, 42), and increased rate of tumor recurrence (43). Retrospective studies have evaluated the correlation between survivin, disease variables, and clinical outcomes. (In Table 1, we have included only those studies that evaluated the correlation between survivin and patient outcomes.) Elevated survivin expression is associated with clinicopathologic variables of aggressive disease and shows a strong correlation with shorter disease-free or overall survival in most studies, identifying it as a significant independent prognostic indicator of poor outcome in patients with most tumor types. The prognostic value of survivin in breast cancer is not clear and studies have shown positive (poor; ref. 44), negative (favorable; ref. 45), or no correlation (46) with clinical outcome. Elevated survivin as a significant indicator of favorable outcome in patients with gastric (47) or non–small-cell lung (48) cancer and osteosarcoma (49) has been reported.

The differences in prognostic value of survivin may reflect differences in the methods used to detect survivin,

nuclear versus cytoplasmic subcellular localization, and/or differential regulation of splice variants with opposing functions. Nuclear survivin expression is an unfavorable prognostic indicator in esophageal, hepatocellular, non–small-cell lung, and ovarian cancers, mantel cell lymphoma and cholangiocarcinoma, and endometrial cancers (37, 50, 51). In contrast, favorable outcome associated with nuclear survivin has been reported for gastric, bladder, and breast cancers, ependymoma, and osteosarcoma (50). Nuclear survivin may regulate cell proliferation whereas cytoplasmic survivin may be involved in cell survival but not cell proliferation (50). Using immunohistochemical analysis, nuclear survivin has been detected with several polyclonal antibodies (Novus, Littleton, CO; Santa Cruz, Santa Cruz, CA; Alpha Diagnostics, San Antonio, TX; Altieri Lab, Worcester, MA). Cytoplasmic survivin has been detected with polyclonal antibodies as well as monoclonal antibodies (Santa Cruz and 8E2 monoclonal antibody developed by the Altieri lab). Predominantly nuclear survivin is detected in ovarian cancer (35, 52) whereas predominantly cytoplasmic survivin is detected in pancreatic cancer (53) using the same Alpha Diagnostics polyclonal antibody. Furthermore, the Novus polyclonal antibody detects predominantly cytoplasmic survivin in oral squamous carcinoma cells (54) but detects predominantly nuclear survivin in patients with laryngeal squamous cell carcinomas (55). These findings suggest that antibody specificity may not be the determinant responsible for the predictive value of survivin localization. Subcellular localization may reflect the amount, transport, or degradation of survivin and its splice variants. Clinical data suggest that loss of the survivin 2B splice variant, which does not seem to possess antiapoptotic activity in limited studies and may antagonize wild-type survivin, is associated with tumor progression. In patients with renal and gastric cancers, survivin 2B expression was lower in later-stage

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tumors (56–58), suggesting that loss of 2B may be associated with tumor progression. The non-antiapoptotic survivin 2B variant predominates in neuroblastomas with good prognosis (59) and was associated with benign brain tumors (60). Survivin ΔEx3 predominates over 2B in patients with acute lymphoblastic leukemia (61). Because the antisurvivin antibodies used in these studies do not distinguish between splice variants with similar and opposing functions, molecular characterization of function and localization of multiple survivin forms may be required to clarify the true prognostic value of survivin and, from a therapeutic standpoint, may help identify optimally selective survivin-targeted strategies. Two additional survivin splice variants, survivin 2α (62) and survivin 3B (63), have been identified. Survivin 2α can bind to survivin and antagonize survivin function, suggesting that survivin 2α may alter the antiapoptotic activity of survivin in tumor cells (62).

Expression of Survivin and an Emerging Role in Regulating Function in Normal Hematopoietic and Immune Cells

Survivin was originally detected only in normal adult thymus and placenta; however, subsequent studies using more sensitive methods have revealed that many adult tissues express survivin (Table 2) albeit at levels lower than cancer cells. The demonstration that survivin levels in normal tissues can be up-regulated by cytokines suggests that survivin may have physiologic roles in regulating proliferation and survival.

Role of Survivin and Cytokine-Regulated Expression in Vascular Endothelial Cells

Apoptosis is believed to be an important factor in vascular remodeling in normal and pathologic conditions. The angiogenic cytokines, vascular endothelial growth factor, basic fibroblast growth factor, and angiopoeitin 1, and hypoxia/reoxygenation regulate normal endothelial cell apoptosis and survival by modulating survivin expression as a consequence of phosphatidylinositol 3-kinase/Akt pathway activation (42, 64, 65). Survivin disruption abrogates the effects induced by angiogenic factors (25), pointing to survivin as a key factor for endothelial cell integrity. Similarly, angiotensin II enhances survival of retinal endothelial cells, which is mediated by up-regulation of survivin through the phosphatidylinositol 3-kinase/Akt pathway and inhibits hyperoxygen-induced retinal regression through survivin in a murine model (66). Apoptosis in human umbilical vascular endothelial cells (HUVEC) is inhibited by angiopoeitin 1 via up-regulation of survivin expression through the forkhead-related transcription factor pathway (67). Interleukin-11 induces survivin expression in HUVEC via the signal transducer and activator of transcription-3 pathway (68) and protects human endothelial cells from graft injury caused by allogenic peripheral blood mononuclear cells (69). Furthermore, hypoxia/ischemia induces survivin expression in microvessels in the peri-infarct regions in mouse brain (70), implicating survivin as a regulator of angiogenesis in ischemic brain. Whereas survivin up-regulation in hypoxic brain was associated with vascular endothelial growth factor expression, induction in cultured endothelial cells is at least partially vascular endothelial growth factor independent (70), suggesting that hypoxia-induced survivin expression is not solely a consequence of vascular endothelial growth factor stimulation. Up-regulation of survivin promoter activity by hypoxia was further enhanced by the hypoxia-responsive element in several human tumor cell lines (71), supporting hypoxia-dependent regulation of survivin. These studies suggest that manipulation of survivin expression in endothelial cells may have therapeutic benefit for diseases in which vascular remodeling or function is deregulated. Recently, survivin was found to be elevated in pulmonary arteries of patients with pulmonary arterial hypertension and in an experimental pulmonary arterial hypertension rat model, which normally induces right ventricular failure and premature death (72). Survivin disruption by inhalation of adenovirus containing dominant-negative T34A-survivin induced pulmonary vascular apoptosis and reversed pulmonary arterial hypertension. Inhibition of apoptosis in endothelial cells by survivin transfer may improve endothelial cell viability and rescue ischemia/hypoxia conditions in the central nervous system or, alternatively, may limit tumor angiogenesis or pathologic vessel remodeling in acute or chronic vascular disorders.

Survivin in Polymorphonuclear Neutrophils

CD34^{+} hematopoietic stem and progenitor cells express high levels of survivin compared with lineage committed CD34^{−} cells or blood mononuclear cells, indicating that survivin expression is down-regulated with hematopoietic cell differentiation (29), reminiscent of survivin down-regulation during development (3). Consistent with this finding, immature neutrophils express survivin (26) whereas mature blood neutrophils do not (26, 29). Interestingly, mature neutrophils can reexpress survivin when stimulated with the neutrophil growth and survival factor granulocyte colony-stimulating factor or granulocyte macrophage colony-stimulating factor *in vitro* or under inflammatory conditions *in vivo* (26). Conversely, administration of antisense survivin oligonucleotides in neutrophils shortens their life span even in the presence of granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, or interleukin-3. Survivin expression was up-regulated in terminally differentiated neutrophils by these cytokines without cell cycle progression, indicating that survivin expression is not restricted to proliferating cells and that survivin can block apoptosis in a cell cycle–independent manner (26). This study also indicates that growth factor–mediated expression of survivin is required to block apoptosis in terminally differentiated neutrophils.

Role of Survivin in T Lymphocytes

Survivin is expressed in thymocytes, splenic T cells, and human adult peripheral blood T lymphocytes, and its expression can be induced by interleukin-2 plus anti-CD3 (29), concanavalin A (7), or phytohemagglutinin (73). The role of survivin in T cells has been extensively

Table 2. Survivin in normal adult tissues

Tissues	Cells	Species	Regulation	Detection method	References
T cells	Peripheral blood adult T cells, thymocytes, memory T cells	Human, mouse	PHA and IL-2, ConA, anti-CD3, OX40	IHC, RT-PCR, FACS, Northern	(4, 7–9, 27, 29, 73)
CD34+ cells	Cord blood, adult marrow CD34+ cells	Human	FL + SCF + Tpo	RT-PCR, Western, FACS	(10, 29)
Neutrophils		Human	GM-CSF or G-CSF	RT-PCR, Western	(26)
Megakaryocytes		Mouse		Immunofluorescence, RT-PCR	(28, 135)
HUVEC		Human	VEGF, angiopoeitin 1, bFGF, IL-11	Northern, Western, luciferase assay	(25, 64, 68, 136)
Arterial muscle	Arterial smooth muscle cells	Rabbit, mouse, rat	Vascular injury, PDGF-AB	IHC, Western	(137)
Liver	Hepatocytes	Mouse	Up-regulated by hepatectomy, Fas antibody, down-regulated by ischemia	RT-PCR, Western	(76–78)
Gastrointestinal tract mucosa	Parietal cells, mucosal epithelial cells	Human		RT-PCR, Western, IHC	(81)
Keratinocytes, melanocytes		Human, mouse	UVB	IHC	(82, 138)
Brain	Neuronal precursor cells, neuron, astrocyte, oligodendrocyte, ependymal cells, chroid plexus	Human, mouse	Hypoxia	RT-PCR, SAGE, *in situ* hybridization	(70, 79, 80)
Endometrium		Human		IHC, RT-PCR	(139)
Cervical mucosa		Human		IHC	(140)
Ovary	Oocytes, stromal cells, and granulosa cells	Human	HCG	Northern, RT-PCR	(85)
Testes	Interstitial Leydig cells, spermatocytes	Human, mouse, rat	SCF	RT-PCR, Western, Northern, IHC	(7, 84, 141)
Breast		Human		RT-PCR	(142)
Spleen		Mouse	Anti-CD3, ConA	Northern	(7)
Placenta		Human		Northern	(4)

Abbreviations: bFGF, basic fibroblast growth factor; ConA, concanavalin A; FACS, fluorescence-activated cell sorting; FL, Flt3 ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; HCG, human chorionic gonadotropin; Northern, Northern blot analysis; PDGF-AB, platelet-derived growth factor AB; PHA, phytohemagglutinin; SAGE, serial analysis of gene expression; SCF, stem cell factor; Tpo, thrombopoietin; VEGF, vascular endothelial growth factor; Western, Western blot analysis.

investigated using T cell–specific survivin knockout mice (9, 27). In mice with survivin deletions occurring at different stages of T-cell development, loss of survivin in lck-Cre; survivin^flox/flox mice at earlier stages induced a defect in thymic development, blocking transition from double-negative to double-positive stages, whereas deletion in CD4-Cre; survivin^flox/flox mice at late stages decreased the number of peripheral blood T cells with no effect on normal thymic development (27). Survivin deficiency did not directly induce T-cell apoptosis but impaired mitogen-induced proliferation and cell cycle progression in adult T cells and homeostatic proliferation of T cells in newborn mice. Similarly, in lck-Cre; survivin^flox/flox mice, loss of survivin blocked transition of thymocytes from double negative to double positive, produced cell cycle arrest at G_1-S and a spindle formation

defect, and increased cell death in proliferating double-negative cells without triggering apoptosis in resting double-negative thymocytes (9). This suggests that survivin regulates mitotic progression but does not directly regulate apoptosis and that the cell death observed in proliferating cells results as a consequence of a defect in cytokinesis and not from loss of survivin. Impaired thymocyte development as a consequence of loss of survivin was not rescued by Bcl-2 or loss of p53, suggesting that survivin regulates thymocyte development via p53- and Bcl-2-independent mechanisms. These data strongly suggest the developmental regulation of survivin during thymocyte differentiation.

Recently, survivin induction by OX40 costimulatory signals was found to be required for effector T-cell proliferation. Survivin expression was induced in peripheral

blood T cells by OX40 during late G_1 whereas blocking survivin inhibited S-phase transition and cell division leading to apoptosis (8). These and other data suggest that survivin regulates G_1-S transition in T lymphocytes (8, 9). In addition, transgenic expression of survivin in thymocytes under the control of the lck promoter induces hyperproliferation in response to phorbol 12-myristate 13-acetate and ionomycin without an effect on apoptosis (74), further supporting a role for survivin in regulating cell cycle progression and proliferation of T cells without affecting apoptosis.

Expression and Potential Role of Survivin in Adult Stem Cells

Mouse embryonic stem cells express survivin (75) and homozygous gene deletion in mice leads to embryonic death at E4.5 (6), indicating that survivin expression is required in totipotent stem cells. Survivin is expressed in normal human CD34$^+$ cells (10, 29) that contain the population of stem cells capable of long-term hematopoietic reconstitution. Hematopoietic growth factors, such as thrombopoietin, stem cell factor, and Flt3 ligand, which stimulate proliferation, cell cycle progression, and survival of CD34$^+$ cells, up-regulate survivin mRNA and protein expression in these cells (10, 29), as well as mRNA for the survivin splice variants ΔEx3 and 2B.[1] In contrast, growth factor deprivation down-regulates survivin expression, which correlates with elevated active caspase-3 and apoptosis (29). Inhibitors of mitogen-activated protein kinase p42/p44 or phosphatidylinositol 3-kinase suppress growth factor–induced survivin expression, suggesting that survivin expression is regulated downstream of mitogen-activated protein kinase p42/p44 and phosphatidylinositol 3-kinase (10). Although its expression is associated with cell cycle progression, survivin was up-regulated in all phases of the cell cycle in CD34$^+$ cells after cytokine stimulation (29), suggesting cell cycle–dependent and –independent regulation, in contrast to the cell cycle–dependent and selective expression during G_2-M in the majority of cancer cells. Using freshly isolated fluorescently labeled G_0 CD34$^+$ cells, survivin was shown to be up-regulated during G_0, before cells enter G_1, and that survivin expression was a function of growth factor stimulation and not of cytokine-driven cell cycle progression (10). Ectopic expression of survivin in mouse bone marrow cells enhances proliferation and cell cycle progression of hematopoietic progenitor cells and inhibits growth factor deprivation–induced apoptosis. Antagonizing survivin by expression of dominant-negative or antisense survivin retroviral constructs inhibited these effects. These findings suggest that survivin regulates hematopoietic progenitor cell proliferation (10). Overexpression of survivin induces hyperproliferation of wild-type hematopoietic progenitor cells, which is absent when the cyclin-dependent kinase (Cdk) inhibitor p21$^{WAF/Cip1}$ is deleted (11). Apoptosis measured by active caspase-3 and hypodiploid DNA

content are reduced by ectopic survivin in p21$^{+/+}$ but not in p21$^{-/-}$ cells. In contrast, elevated S phase induced by ectopic survivin is unaffected by p21 status, indicating that survivin blocks apoptosis of hematopoietic progenitor cells via a p21-dependent mechanism but promotes S phase in a p21-independent manner. In addition, aberrant survivin expression induces polyploidy in progenitor cells, which is enhanced in the absence of p21, suggesting a functional link between survivin and p21 in regulating hematopoietic cell division (11). We have now confirmed a specific role for survivin in regulating normal adult hematopoietic stem and progenitor cells using tamoxifen inducible Cre-ER^{TM}; survivin$^{flox/flox}$ mice. Survivin gene deletion by tamoxifen *in vivo* significantly reduces total progenitor cells and primitive hematopoietic stem cells in the bone marrow [41 ± 6% reduction of granulocyte-macrophage colony-forming units, 59 ± 9% reduction in Sca-1$^+$, c-kit$^+$, lineage-negative (SKL) cells, and 64 ± 9% reduction in CD34$^-$SKL cells], with only minimal effect on mature blood cells.[1] These data are consistent with the developmental down-regulation of survivin with hematopoietic differentiation (29) and strongly suggest that survivin plays a physiologic role in maintaining normal adult hematopoiesis through regulation of the most primitive hematopoietic stem cells.

Survivin in Erythroid and Megakaryocyte Development

Survivin is differentially expressed during erythroid versus megakaryocyte development (28). Survivin is expressed in maturing erythroid cells whereas murine megakaryocytes express ∼4-fold lower levels of survivin mRNA and no detectable protein. Overexpression of survivin in murine bone marrow cells led to decreased production of megakaryocytes and blocked their terminal maturation and polyploidization. In contrast, siRNA for survivin or haploinsufficiency of the survivin gene decreased erythroid cell expansion without affecting megakaryocytes. Survivin deficiency severely impaired production of mouse bone marrow erythroid (erythroid blast-forming units) or megakaryocytic (megakaryocyte colony-forming units) colonies *in vitro* (28). These findings suggest that, like myeloid progenitor cells, survivin expression is required in megakaryocytes and erythroid progenitor cells and that survivin plays a significant role in erythropoiesis. Interestingly, survivin down-regulation is an essential component of megakaryocyte maturation and thus may play a role in platelet formation (28).

Survivin in Other Adult Tissues

Survivin expression is detected in adult liver and is down-regulated by ischemia (76) but up-regulated by hepatectomy (77). Furthermore, the Fas agonistic antibody Jo2 induces survivin expression in liver whereas survivin haploinsufficiency sensitizes hepatocytes to Jo2 antibody–mediated apoptosis via the mitochondrial pathway (78), indicating that hepatocyte proliferation and apoptosis are regulated by survivin. Survivin is expressed in neurons, astrocytes, oligodendrocytes, ependymal cells, and chroid plexus in the human brain (79). In a mouse hypoxia model, survivin expression was significantly up-regulated in

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[1] Unpublished data.

neurons (70). Conditional survivin deletion in neuronal precursor cells using a Cre-loxP system showed significant apoptosis in cerebrum, cerebellum, brainstem, spinal cord, and retina, indicating that survivin functions as an antiapoptotic protein in neuronal development *in vivo* (80). Survivin is expressed in gastrointestinal tract mucosa in humans, which, like the hematopoietic system, undergoes continuous cell renewal (81). This suggests that survivin may be important in regulating self-renewal and differentiation of crypt stem cells. Survivin expression has also been reported in melanocytes (82), keratinocytes (83), testes (84), and ovary (85) in humans. Stem cell factor and human chorionic gonadotropin also induce survivin expression in testes (84) and in ovarian granulosa cells (85), suggesting that survivin may have a role in the regulation of spermatogenesis and oogenesis.

Differences in Survivin Expression and Function between Cancer and Normal Tissues

Although survivin is expressed and regulated in normal tissues characterized by self-renewal and proliferation, its expression is significantly lower than in transformed cells. This raises the question of mechanisms responsible for survivin up-regulation in cancer tissues. Survivin expression may be higher, simply because cancer cells are proliferating faster. Although this undoubtedly contributes to survivin levels in many cancers, survivin expression is also deregulated in Ki-67-negative MCF-7 breast cancer cells (3), suggesting that survivin expression may not be a direct consequence of cell proliferation. The intracellular pathways that activate survivin transcription or block survivin sequestration may be more active in malignant than in normal tissues. DNA-protein interaction in the survivin promoter is distinct from nuclear proteins isolated from normal cells and cancer cells, suggesting differences in regulation of survivin expression (86). Oncogenes such as Bcr-abl (87) and activated H-Ras (88), which are absent in normal tissues, can significantly increase survivin expression. Survivin expression is associated with signal transducer and activator of transcription-3 activation in gastric cancer (89), breast cancer (90), and primary effusion lymphoma (91). Wild-type p53 (92, 93) and retinoblastoma (94) can transcriptionally repress survivin; however, p53 and retinoblastoma are mutated and inactivated in variety of cancer cells. In addition, E2F activators (E2F1, E2F2, and E2F3) can induce survivin transcription, suggesting that the retinoblastoma/E2F and p53 pathways may contribute to aberrant survivin expression (94). Nuclear factor κB is also involved in transcriptional up-regulation of survivin in B-cell lymphoma (95). Recently, a mutation was found in the survivin promoter that correlates with overexpression of survivin mRNA in cancer cells (96). Survivin expression in acute myelogenous leukemia (AML) cells, like normal cells, is regulated by hematopoietic cytokines (97); however, AML cells often coexpress cytokines and their receptors, suggesting that survivin may also be elevated by autocrine or paracrine mechanisms. Interaction of survivin with heat shock protein 90 blocks survivin degradation whereas disruption of their association induces proteosomal survivin degradation, apoptosis, and mitotic defects in HeLa cells (98), which suggests that blocking survivin sequestration may be an additional mechanism accounting for elevated survivin expression in cancers.

Survivin is up-regulated during G_0-G_1 phase in growth factor–stimulated $CD34^+$ cells (10, 29), during late G_1 in OX40-stimulated T cells independent of mitotic progression (8), and in nonproliferating terminally differentiated human neutrophils by cytokines (26). Moreover, ectopic survivin increases the number of hematopoietic progenitor cells in S phase (10, 11) whereas survivin disruption induces G_1-S arrest in T cells and reduces S phase in hematopoietic progenitor cells (8–10). These findings point to a role for survivin as a regulator of G_1-S transition in some normal tissues. Although survivin mediates the antiapoptotic activity of granulocyte colony-stimulating factor or granulocyte macrophage colony-stimulating factor in terminally differentiated noncycling neutrophils, the role of survivin in blocking apoptosis during G_0-G_1 in other cell types is not clear. In contrast to normal cells, expression of survivin during G_0-G_1 and cell cycle arrest at G_1-S following survivin disruption do not seem to be common in most cancer cells. Disruption of survivin or inhibition of heat shock protein 90 in HeLa cells failed to cause G_1-S arrest (98). This may be due to inactivation of retinoblastoma or p53 in HeLa cells (98) or selective expression of survivin during mitosis in cancer cells. However, survivin expression in Ki-67-negative breast cancer cells (3), retinoblastoma phosphorylation by survivin resulting from its interaction with $Cdk4/p16^{INK4a}$ and activation of Cdk2/cyclin E complex in hepatoma cells (12), and resistance to vitamin D–mediated G_1 arrest associated with increased S + G_2-M phase by ectopic survivin in MCF7 breast cancer cells (14) suggest that survivin may also regulate G_1-S transition in some cancer cells.

The mechanisms whereby survivin regulates cancer cell proliferation is poorly understood; however, survivin can regulate apoptosis, cell cycle, or cytokinesis through functional or physical interactions with heat shock protein 90 (98), Smac/Diablo (99), X-linked inhibitor of apoptosis protein (99), $p21^{WAF1/Cip1}$ (5, 12), Cdk4 (12), Cdc2 (Cdk1; ref. 3), retinoblastoma/E2F (94), nuclear factor κB (95), signal transducers and activators of transcription-3 (89–91), or p53 (1, 92, 93). It is important therefore to determine whether survivin regulates normal cell proliferation using the same pathways. In normal hematopoietic cells, survivin regulates apoptosis through p21-dependent pathways (11), which is consistent with suppression of apoptosis in hepatoma cells by interaction of survivin with the procaspase-3/p21 complex (12). Disruption of survivin can up-regulate and activate p53 in T cells (9) and in breast cancer cells (100), findings consistent in both cancer and normal cells. Induction of apoptosis in hematopoietic progenitor cells (11), HUVEC (16), and in several cancers cells (3) by the phosphorylation dead T34A-survivin mutant suggests that phosphorylation of survivin on

Thr[34] by Cdc2 is required for survival in both normal and cancer cells. Mitochondrial survivin exerts cytoprotection of human cancer cells by preventing activation of caspase-9 and promotes anchorage-independent growth (101). Survivin is not found in mitochondria in normal tissues, suggesting that mitochondrial survivin is exclusively associated with tumor transformation (101). Continued evaluation of the differences in mechanism of action of survivin between cancer and normal cells will likely prove important for the development of selective and minimally toxic antisurvivin therapies.

In vivo Interventions Using Antisurvivin Strategies

The robust expression of survivin in cancer versus normal cells (4), resistance to apoptosis induced by various chemotherapeutic agents as a consequence of survivin expression (39, 40), the correlation of survivin with poor prognosis (51, 102) and resistance to therapy (41, 42), and survivin induction by anticancer agents (17) suggest that survivin is an inducible resistance factor in cancer cells and involved in the emergence of refractory phenotype to anticancer therapies. These findings have led to analysis of whether survivin disruption can sensitize cancer cells to subsequent therapeutic interventions. Several preclinical studies have shown that disrupting survivin expression or function in cancer cells decreases their proliferation and enhances apoptosis. These include suppressing survivin expression by antisense, ribozyme, siRNA, or shRNA approaches or antagonizing survivin function by dominant-negative survivin or by Cdk inhibitors. Antisurvivin therapy has been evaluated in several preclinical models using mice harboring preestablished tumors (Table 3). In a breast cancer model in mice, intratumor injection of adenovirus expressing T34A-survivin produced significant reduction of preestablished tumor size with an increase in apoptotic cells (15). T34A-survivin injection into disseminated breast cancer cells in the peritoneal cavity of severe combined immunodeficient mice significantly reduced tumor growth. Interestingly, infection of T34A-survivin into proliferating normal human fibroblasts, endothelium (HUVEC), or smooth muscle cells did not affect cell viability *in vitro* and no systemic toxicity was noted in mice treated with T34A-survivin adenovirus, leading to the conclusion that targeting survivin by adenovirus may provide selectivity for tumor cells and limited toxicity for normal tissues *in vivo*. A subsequent study showed that inhibition of the T34 phosphorylation site of survivin by the Cdk inhibitor flavopiridol enhanced breast cancer cell apoptosis in mice without evidence of organ toxicity (17). Disruption of survivin function by T34A-survivin via adenovirus-mediated injection into mice bearing breast cancers induced tumor cell–derived endothelial cell apoptosis, in addition to tumor cell apoptosis (16), providing another rationale for survivin disruption as a means to block tumor neovascularization. However, T34A-survivin also induced apoptosis in HUVEC *in vitro* (16), in contrast to a lack of apoptotic effect on HUVEC in earlier reports (15), raising the question of whether survivin disruption may have toxicity towards normal blood vessel development, not just against neovascularization in tumors. Other studies targeting survivin *in vivo* have also shown positive results. Intratumoral injection of adenovirus expressing antisense or T34A-survivin into prostate cancers in mice significantly inhibited tumor growth (18, 19) and enhanced antiandrogen sensitivity (18). Injection of shRNA for survivin into rhabdomyosarcomas (22) resulted in inhibition of tumor cell growth, and injection of dominant-negative C84A-survivin in adeno-associated virus into colon cancers inhibited tumor cell growth and angiogenesis in mice without obvious organ toxicity (23). Adenoviral survivin siRNA significantly inhibited glioma cell growth in xenografted mice (20) and injection of antisense or C84A-survivin into large-cell lymphomas reduced tumor cell growth and enhanced tumor-specific CTL-mediated cell death (21). These studies using *in vivo* antisurvivin therapy clearly indicate that disrupting survivin in cancers may be clinically beneficial. More recently, a small peptide, shepherdin, which blocks the interaction of heat shock protein 90 with survivin, has been developed. Proliferation of normal human fibroblasts, granulocyte-macrophage colony-forming units, and granulocyte erythrocyte macrophage megakaryocyte colony-forming units derived from CD34^{+} cells was not significantly affected at concentrations of shepherdin sufficient to reduce tumor cell viability *in vitro*, although at higher concentration, some inhibition of hematopoietic progenitor cells was seen, at least *in vitro* (24). *In vivo*, sheperdin showed significant reduction of human breast and prostate cancer cell growth without apparent toxicity in a mouse xenograft model (24). These elegant and encouraging studies provide strong evidence for efficacy of survivin-targeted therapy and address issues of normal organ toxicities.

Potential Adverse Effects of Antisurvivin Therapy

Although the studies described above have all shown efficacy of antisurvivin therapies for cancers *in vivo* without obvious toxicity and strongly support clinical evolution of antisurvivin therapies, a potential reason for lack of obvious side effects may relate to the minimal systemic dissemination of antisurvivin reagents due to local intratumor administration or could be due to the level of survivin between cancer cells and normal cells or the time frame of analysis. Alternatively, antisurvivin therapies may not affect most nonproliferating adult tissues because survivin may be required only for proliferating adult tissues, such as hematopoietic cells or T lymphocytes. Whereas a minimal effect on the number of mature myeloid hematopoietic progenitor cells derived from human peripheral blood CD34^{+} cells was observed by shepherdin at a dose sufficient to almost completely suppress growth of the tumor cells *in vitro*, precursor erythroid blast-forming units were inhibited (24) and, at slightly higher shepherdin concentrations, granulocyte-macrophage colony-forming units and granulocyte erythrocyte macrophage megakaryocyte colony-forming units were also dose-dependently inhibited (24).

Table 3. Antisurvivin strategies and interventions

Disease model	Strategy	Effect	Toxicity	Reference
Breast cancer xenograft and i.p. disseminated breast cancers	Intratumoral infection of T34A survivin using adenovirus	Inhibition by 40% of established tumor growth and reduced i.p. tumor dissemination	No effect on normal proliferating cells *in vitro* and endothelial cells and no systemic toxicity	(15)
Breast cancer xenograft	Intratumoral infection of T34A survivin using adenovirus	Inhibition of preestablished tumors and triggering of apoptosis; ~60% reduction of tumor-derived blood vessels		(16)
Breast cancer xenograft	Suppression of T34 survivin phosphorylation by flavopiridol	Inhibition of preestablished breast cancers and increased recipient mice survival	No systemic toxicity	(17)
Prostate cancer xenograft	Intratumoral infection of T34A survivin using adenovirus	Increased sensitivity to antiandrogen therapy		(18)
Prostate cancer xenograft	Intratumoral injection of antisense survivin using adenovirus	Growth inhibition of tumors	No systemic toxicity	(19)
Glioma xenograft	Intratumoral injection of siRNA for survivin using adenovirus	Suppression of tumor growth		(20)
Large-cell lymphoma	Intratumoral injection of antisense survivin or C84A survivin plasmid plus tumor-specific CTL	Growth inhibition of established tumors		(21)
Rhabdomyosarcoma xenograft	Intratumoral injection of shRNA for survivin	>70% reduction in tumor growth		(22)
Colon cancer xenograft	Intratumoral injection of C84A survivin using adeno-associated virus	Induction of apoptosis, inhibition of angiogenesis and tumor growth	No obvious cytotoxicity	(23)
Breast cancer xenograft, prostate cancer xenograft	Systemic administration of peptide blocking survivin and heat shock protein 90 interaction	Growth inhibition of tumors	No effect on normal cells *in vitro*; no overt systemic toxicity	(24)

Consistent with these findings, ablation of survivin reduces the number of granulocyte-macrophage colony-forming units, erythroid blast-forming units, and megakaryocyte colony-forming units (10, 11, 28), as well as SKL cells and CD34⁻SKL cells, which contain primitive long-term repopulating cells (103, 104). These data suggest that prolonged or intensified antisurvivin therapy may have the potential to affect primitive hematopoietic cells. Because only hematopoietic stem cells support hematopoiesis, not progenitor cells (105, 106), evaluation of the *in vivo* function of hematopoietic stem cells treated with antisurvivin therapy using long-term repopulating assays will be required to provide accurate information on primitive hematopoietic cell toxicity.

In addition to hematopoietic stem cells, impaired differentiation, cell cycle progression, or survival upon disruption of survivin in T cells has been reported (8, 9, 27). These findings suggest that antisurvivin therapy could potentially affect T cells that could otherwise participate in elimination of cancer cells. Neutropenia and thrombocytopenia are two major complications related to most

anticancer therapies. Deletion of survivin in mature neutrophils suppresses survival induced by granulocyte colony-stimulating factor or granulocyte macrophage colony-stimulating factor (26) and because survivin expression is required for megakaryocyte progenitor cell proliferation (28), survivin disruption may accelerate neutropenia and/or thrombocytopenia. In light of new information that other normal adult tissues also express survivin (i.e., central nervous system, uterus, testes, ovary, liver, gastrointestinal tract mucosa, keratinocytes, and myocardium), major organ systems, particularly those characterized by self-renewal, should be carefully monitored for toxicity over time.

Concluding Remarks

Recent studies using molecular dissection of genes associated with aberrant proliferation of cancer cells and endothelial cells have identified survivin as a candidate gene responsible for cancer progression and vascular disease and as an attractive molecular therapeutic target

for these pathologic conditions. As emphasized in this review, survivin is not a cancer-specific molecule but is also involved in regulating normal cell function, which suggests that survivin disruption could affect normal cell function, particularly the hematopoietic and immune systems. Antisurvivin therapies developed to date have not revealed major systemic toxicities in animal models and are extremely encouraging. Continuing investigations of mechanisms of differentially regulating survivin expression and function in tumor and normal cells will help to pinpoint crucial differences in survivin behavior that can be used to develop additional innovative strategies for selectively antagonizing survivin.

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