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 G1-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,
| Cancer type (no. patients) | Method* | Form | Site 1 | Survivin Correlation with elevated survivin | Clinicopathologic variables | Survival 1 | Reference |
|---------------------------|---------|------|-------|---------------------------------------------|-----------------------------|-----------|-----------|
| 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                          | NC        | (113)     |
| Ovarian                   |         |      |       |                                             |                             |           |           |
| (110) IHC                 | WT      | N    |       | NC                                          |                             | NC        | (114)     |
| (32) IHC                  | WT      | N    | Stage |                                             |                             | ↓         | (52)      |
| (103) IHC                 | WT      | N    | Residual disease                           | NC                          | ↑         | (35)      |
| (49) IHC                  | WT      | N    | Grade; histology                           | NC                          | ↓         | (115)     |
| (124) IHC                 | WT      | NC   | resistance to taxanes                      | NC                          | NC        | (41)      |
| Central nervous system    |         |      |       |                                             |                             |           |           |
| (59; meduloblastoma)      | IHC     | WT   | N     | Aggressiveness                              |                             | ↓         | (116)     |
| (92; glioblastoma)        | WB      | WT   | Trend-histology; treatment resistance      |                             | ↓         | (117)     |
| (43; astrocytne 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      | N    | NC    |                                             |                             | ↑         | (119)     |
| (171) IHC                 | WT      | C    | NC    |                                             |                             | ↓         | (39)      |
| (49) IHC                  | WT      | C    | NC    |                                             |                             | ↑         | (120)     |
| (96) IHC                  | WT      | C    | Histology                                   | NC                          | NC        | (121)     |
| Melanoma                  |         |      |       |                                             |                             |           |           |
| (36; sentinel nodes)      | RT-PCR  | WT   | N     |                                             |                             | ↓         | (122)     |
| Gastric                   |         |      |       |                                             |                             |           |           |
| (133) IHC                 | WT      | N    | NC    |                                             |                             | ↓         | (47)      |
| Sarcoma                   |         |      |       |                                             |                             |           |           |
| (89) RT-PCR               | WT      | N    |       | Grade; aggressiveness                       |                             | ↓         | (123)     |
| (63) ELISA; WB            | WT      | DeltaEx3 N | 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                                          | Site                       | ↓         | (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.

1Nuclear (N) versus cytoplasmic (C) staining.

2Shorter (↓) or prolonged (↑) disease-free (DF) or overall (OS) survival.

3NC, no statistical correlation.

(Continued on the following page)
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

| 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 | Grade; stage | ↓                        | (38)    |
| (51)                     | RT-PCR; WB; WT | NC             | ↓                        | (129)   |
| Hematologic               |             |          | Low WBC; FAB M2 subtype | ↓ (trend) | (132)  |
| AML (45; pediatric)       | WB; FC; WT; N | NC                | ↓                        | (130)   |
| AML (116; adult)          | WB; WT; N   | NC                | NC                       | (131)   |
| AML (125)                 | IHC; WT; C  | Low WBC; FAB M2 subtype | ↓ (trend) | (132)  |
| AML (31; adult)           | RT-PCR; WT; N | NC             | ↓                        | (33)    |
| ALL (16)                  | RT-PCR; WT; N | NC             | ↓                        | (133)   |
| Large-cell lymphoma       | IHC; WT; C  | NC                | ↓                        | (134)   |
| B-cell lymphoma           | IHC; WT; N  | NC                | ↓                        |          |

Note: IHC = immunohistochemistry; WT = western blotting; FC = fluorescent cell sorting; WB = western blotting; Q-RT-PCR = quantitative reverse transcriptase PCR; RT-PCR = reverse transcriptase PCR.
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 angiotropin 1, and hypoxia/reoxygenation regulate normal endothelial cell apoptosis and survival by modulating survivin expression as a consequence of phosphotidylinositol 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 phosphotidylinositol 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 downregulation 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
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; survivinflox/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 G1-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

### Table 2. Survivin in normal adult tissues

| Tissues                      | Cells                                      | Species     | Regulation                     | Detection method         | References |
|------------------------------|--------------------------------------------|-------------|--------------------------------|--------------------------|------------|
| T cells                      | Peripheral blood                           | 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                         | Human       | FL + SCF + Tpo                 | RT-PCR, Western, FACS    | (10, 29)   |
| Neutrophils                  | Marrow CD34+ cells                        | Human       | GM-CSF or G-CSF                | RT-PCR, Western          | (26)       |
| Megakaryocytes               |                                            | Mouse       |                                | Immunofluorescence, RT-PCR| (28, 135)  |
| HUVEC                        |                                            | Human       | VEGF, angiopoietin 1, bFGF, IL-11 | Northern, Western, luciferase assay | (25, 64, 68, 136) |
| Arterial muscle              | Arterial smooth                            | 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, mucus epithelial cells     | Human       |                                | RT-PCR, Western, IHC     | (81)       |
| Keratinocytes, melanocytes   |                                            | Human       | UVB                            | IHC                       | (82, 138)  |
| Brain                        | Neuronal precursor cells, neuron, astrocyte, oligodendrocyte, ependymal cells, choroid plexus | Human, mouse | Hypoxia                        | RT-PCR, SAGE, in situ hybridization | (70, 79, 80) |
| Endometrium                  |                                            | Human       |                                | IHC, RT-PCR, Northern    | (139)      |
| Cervical mucosa              |                                            | Human       |                                | IHC                       | (140)      |
| Ovary                        | Oocytes, stromal cells, and granulosa cells| Human       | HCG                            | RT-PCR, Northern          | (85)       |
| Testes                       | Interstitial Leydig cells, spermatocytes   | Human, mouse, rat | SCF                          | RT-PCR, Western, Northern, IHC | (7, 84, 141) |
| Breast                       |                                            | Human       | Anti-CD3, ConA                 | Northern                  | (142)      |
| Spleen                       |                                            | Mouse       |                                | 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.
blood T cells by OX40 during late G1 whereas blocking survivin inhibited S-phase transition and cell division leading to apoptosis (8). These and other data suggest that survivin regulates G1-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 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 G2-M in the majority of cancer cells. Using freshly isolated fluorescently labeled G0 CD34+ cells, survivin was shown to be up-regulated during G0, before cells enter G1, 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 p21WAF/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-ERTM; survivinflox/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 downregulation 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

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 proteasomal 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 G0-G1 phase in growth factor–stimulated CD34+ cells (10, 29), during late G1 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 G1-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 G1-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 G0-G1 in other cell types is not clear. In contrast to normal cells, expression of survivin during G0-G1 and cell cycle arrest at G1-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 G1-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/p16INK4a and activation of Cdk2/cyclin E complex in hepatoma cells (12), and resistance to vitamin D–mediated G1 arrest associated with increased S + G2-M phase by ectopic survivin in MCF7 breast cancer cells (14) suggest that survivin may also regulate G1-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), p21WAF1/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\textsuperscript{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 anticaner 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 pre-established 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, injection of T34A-survivin into proliferating normal human fibroblasts, endothelium (HUVEC), or smooth muscle cells did not affect cell viability \textit{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 \textit{in vivo}. A subsequent study showed that injection 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 \textit{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 \textit{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 adenocarcinoma and PC3 cells in 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 \textit{in vivo} antisurvivin therapy clearly indicate that disrupting survivin in cancers may be clinically beneficial. More recently, a small peptide, sheperdin, 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 sheperdin sufficient to reduce tumor cell viability \textit{in vitro}, although at higher concentrations, some inhibition of hematopoietic progenitor cells was seen, at least \textit{in vitro} (24). In \textit{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 \textit{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 sheperdin at a dose sufficient to almost completely suppress growth of the tumor cells \textit{in vitro}, precursor erythroid blast-forming units were inhibited (24) and, at slightly higher sheperdin concentrations, granulocyte-macrophage colony-forming units and granulocyte erythrocyte macrophage megakaryocyte colony-forming units were also dose-dependently inhibited (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+CD0 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 vitro 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.

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**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)      |

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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.

References

1. Altieri DC. Survivin, versatile modulation of cell division and apoptosis in cancer. Oncogene 2003;22:8581 – 9.
2. Li F, Ambrosini G, Chu EW, et al. Control of apoptosis and mitotic spindle checkpoint by survivin. Nature 1998;396:580 – 4.
3. Altieri DC. Validating survivin as a cancer therapeutic target. Nat Rev Cancer 2003;3:46 – 54.
4. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 1997;3:817 – 21.
5. Li F, Ackermann EJ, Bennett CF, et al. Pleiotropic cell division defects and apoptosis induced by interference with survivin function. Nat Cell Biol 1999;1:461 – 6.
6. Uren AG, Wong L, Pakusch M, et al. Survivin and the inner centromere protein INCENP show similar cell-cycle localization and gene knockout phenotype. Curr Biol 2000;10:1319 – 28.
7. Kobayashi K, Hatano M, Otaki M, et al. Expression of a murine homologue of the inhibitor of apoptosis protein is related to cell proliferation. Proc Natl Acad Sci U S A 1999;96:1457 – 62.
8. Song J, So T, Cheng M, et al. Sustained survivin expression from OX40 costimulatory signals drives T cell clonal expansion. Immunity 2005;22:621 – 31.
9. Okada H, Bakal C, Shahinian A, et al. Survivin loss in thymocytes triggers p53-mediated growth arrest and p53-independent cell death. J Exp Med 2004;199:399 – 410.
10. Fukuda S, Foster RG, Porter SB, Pelus LM. The antiapoptosis protein survivin is associated with cell cycle cell with normal cord blood CD34(+) cells and modulates cell cycle and proliferation of mouse hematopoietic progenitor cells. Blood 2002;100:2463 – 71.
11. Fukuda S, Mantel CR, Pelus LM. Survivin regulates hematopoietic progenitor cell proliferation through p21WAF1/Cip1-dependent and -independent pathways. Blood 2004;103:120 – 7.
12. Suzuki A, Shiraki K. Tumor cell ‘‘dead or alive’’: caspase and survivin regulate cell death, cell cycle and cell survival. Histol Histopathol 2001;16:583 – 93.
13. Suzuki A, Hayashida M, Ito T, et al. Survivin initiates cell cycle entry by the competitive interaction with Cdk4/p16INK4a and Cdk2/cyclin E complex activation. Oncogene 2000;19:3225 – 34.
14. Li F, Ling X, Huang H, et al. Differential regulation of survivin expression and apoptosis by vitamin D3 compounds in two isogenic MCF-7 breast cancer cell sublines. Oncogene 2005;24:1385 – 95.
15. Mesri M, Wall NR, Li J, et al. Cancer gene therapy using a survivin mutant adenovirus. J Clin Invest 2001;108:981 – 90.
16. Blanc-Brude OP, Mesri M, Wall NR, et al. Therapeutic targeting of the survivin pathway in cancer: initiation of mitochondrial apoptosis and suppression of tumor-associated angiogenesis. Clin Cancer Res 2003;9:2683 – 92.
17. Wall NR, O’Connor DS, Plescia J, et al. Suppression of survivin phosphorylation on Thr34 by flavopiridol enhances tumor cell apoptosis. Cancer Res 2003;63:230 – 5.
18. Zhang M, Latham DE, Delaney MA, Chakravarti A. Survivin mediates resistance to antiandrogen therapy in prostate cancer. Oncogene 2005;24:2474 – 82.
19. Hayashi N, Asano K, Suzuki H, et al. Adenoviral infection of survivin antisense sensitizes prostate cancer cells to etoposide in vivo. Prostate 2005;65:10 – 9.
20. Uchida H, Tanaka T, Sasaki K, et al. Adenovirus-mediated transfer of siRNA against survivin induced apoptosis and attenuated tumor cell growth in vitro and in vivo. Mol Ther 2004;10:162 – 71.
21. Kanwar JR, Shen WP, Kanwar RK, et al. Effects of survivin antagonists on growth of established tumors and B7-1 immunogenic therapy. J Natl Cancer Inst 2001;93:1541 – 52.
22. Caldas H, Holloway MP, Hall BM, et al. Survivin-directed RNA interference cocktail is a potent suppressor of tumor growth in vivo. J Med Genet 2006;43:119 – 28. Epub 2005 May 20.
23. Tu SP, Cui JT, Liston P, et al. Gene therapy for colon cancer by adeno-associated viral vector-mediated transfer of survivin Cys84Ala mutant. Gastroenterology 2005;128:361 – 75.
24. Plescia J, Salz W, Xia F, et al. Rational design of shepherdin, a novel anticancer agent. Cancer Cell 2005;7:457 – 68.
25. Mesri M, Morales-Ruiz M, Ackermann EJ, et al. Suppression of vascular endothelial growth factor-mediated endothelial cell protection by survivin targeting. Am J Pathol 2001;158:1757 – 65.
26. Altznauer F, Martinielli S, Youssef S, et al. Inflammation-associated cell cycle-independent block of apoptosis by survivin in terminally differentiated neutrophils. J Exp Med 2004;199:1343 – 54.
27. Xing Z, Conway EM, Kang C, Winoto A. Essential role of survivin, an inhibitor of apoptosis protein, in T cell development, maturation, and homeostasis. J Exp Med 2004;199:69 – 80.
28. Gurbuxani S, Xu Y, Keerthivasan G, et al. Differential requirements for survivin in hematopoietic cell development. Proc Natl Acad Sci U S A 2005;102:11480 – 5.
29. Fukuda S, Pelus LM. Regulation of the inhibitor-of-apoptosis family member survivin in normal cord blood and bone marrow CD34(+) cells by hematopoietic growth factors: implication of survivin expression in normal hematopoiesis. Blood 2001;98:2091 – 100.
30. Cong XL, Han ZC. Survivin and leukemia. Int J Hematol 2004;80:232 – 8.
31. Granziero L, Ghia P, Ciricosta P, et al. Survivin is expressed on CD40 stimulation and interfaces proliferation and apoptosis in B-cell chronic lymphocytic leukemia. Blood 2001;97:2777 – 83.
32. Badran A, Yoshida A, Wano Y, et al. Expression of the anti-apoptotic gene survivin in myelodysplastic syndrome. Int J Oncol 2003;22:59 – 64.
33. Mori A, Wada H, Nishimura Y, et al. Expression of the antiapoptosis gene survivin in human ovarian carcinoma. Oncol Rep 2002;6:127–34.
34. Tanaka K, Iwamoto S, Gon G, et al. Expression of survivin and its correlation with cell proliferation and prognosis in epithelial ovarian tumors. Int J Gynecol Cancer 2002;12:315–20.
35. Takai N, Miyazaki T, Nishida M, et al. Survivin expression correlates with clinical stage, histological grade, invasive behavior and survival rate in endometrial carcinoma. Cancer Lett 2002;184:105 – 16.
36. Fields AC, Cotsonis G, Sexton D, et al. Survivin expression in hepatocellular carcinoma: correlation with proliferation, prognostic parameters, and outcome. Mod Pathol 2004;17:1378 – 85.
37. Morinaga S, Nakamura Y, Ishiwa N, et al. Expression of survivin mRNA associates with apoptosis, proliferation and histologically aggressive features in hepatocellular carcinoma. Oncol Rep 2004;12:1189 – 94.
38. Kawasaka H, Altieri DC, Lu CD, et al. Inhibition of apoptosis by survivin predicts shorter survival rates in colorectal cancer. Cancer Res 1998;58:5071 – 4.
39. Tanaka K, Iwamoto S, Gon G, et al. Expression of survivin and its relationship to loss of apoptosis in breast carcinomas. Clin Cancer Res 2000;6:127 – 34.
40. Zaffaroni N, Pennati M, Colega L, et al. Expression of the antiapoptotic gene survivin correlates with taxol resistance in human ovarian cancer. Cell Mol Life Sci 2002;59:1406 – 12.
41. Tran J, Master Z, Yu JL, et al. A role for survivin in chemoresistance of endothelial cells mediated by VEGF. Proc Natl Acad Sci U S A 2002;99:4349 – 54.
42. Swana HS, Grossman D, Anthony JN, et al. Tumor content of the antiapoptosis molecule survivin and recurrence of bladder cancer. N Engl J Med 1999;341:452 – 3.
44. Span PN, Sweep FC, Wieringer ME, et al. Survivin is an independent prognostic marker for risk stratification of breast cancer patients. Clin Chem 2004;50:1986–93.
45. Kennedy SM, O’Driscoll L, Purcell R, et al. Prognostic importance of survivin in breast cancer. Br J Cancer 2003;88:1077–83.
46. O’Driscoll L, Linehan R, Kennedy M, et al. Lack of prognostic significance of survivin, survivin-ⅢA, survivin-ⅡB, galectin-3, bag-1, bax-α and MRP-1 mRNAs in breast cancer. Cancer Lett 2003;201:225–36.
47. Okada E, Murai Y, Matsui K, et al. Survivin expression in tumor cell nuclei is predictive of a favorable prognosis in gastric cancer patients. Cancer Lett 2001;163:109–16.
48. Vischioni B, van der Valk P, Span SW, et al. Nuclear localization of survivin is a positive prognostic factor for survival in advanced non-small cell lung cancer. Ann Oncol 2004;15:1654–60.
49. Trieb K, Lehner R, Stulnig T, et al. Survivin expression in human osteosarcoma is a marker for survival. Eur J Surg Oncol 2003;29: 379–82.
50. Li F, Yang J, Ramnath N, et al. Nuclear or cytoplasmic expression of survivin: what is the significance? Int J Cancer 2005;114:509–12.
51. Shinohara ET, Gonzalez A, Massion PP, et al. Nuclear survivin predicts recurrence and poor survival in patients with resected nonsmall cell lung carcinoma. Cancer 2005;103:1685–92.
52. Yoshida H, Ishiko O, Sumi T, et al. Survivin, bcl-2 and matrix metalloproteinase-2 enhance progression of clear cell- and serous-type ovarian carcinomas. Int J Oncol 2001;19:537–42.
53. Kamei K, Doi R, Koizumi M, et al. Survivin expression is a prognostic marker in pancreatic cancer patients. Surgery 2004;136:1443–8.
54. Lo ML, Pannone G, Staibano S, et al. Survivin expression in oral squamous cell carcinoma. Br J Cancer 2003;89:2244–8.
55. Pizem J, Cor A, Gale N. Survivin expression is a negative prognostic marker in laryngeal squamous cell carcinoma and is associated with p53 accumulation. Histopathology 2004;45:180–6.
56. Mahotka C, Krieg T, Krieg A, et al. Distinct accumulation. Histopathology 2004;45:180–6.
57. Meng H, Lu C, Mabuchi H, Tanigawa N. Prognostic significance and variants ingastriccarcinomas: firstclues toarole ofsurvivin-2Bin tumour patterns of survivin splice variants in renal cell carcinomas. Int J Cancer 2004;109:234–41.
58. Yoshida H, Ishiko O, Sumi T, et al. Survivin, bcl-2 and matrix metalloproteinase-2 enhance progression of clear cell- and serous-type ovarian carcinomas. Int J Oncol 2001;19:537–42.
59. Kamei K, Doi R, Koizumi M, et al. Survivin expression is a prognostic marker in pancreatic cancer patients. Surgery 2004;136:1443–8.
60. Lo ML, Pannone G, Staibano S, et al. Survivin expression in oral squamous cell carcinoma. Br J Cancer 2003;89:2244–8.
61. Funayama Y, Kuroiwa T, Nakagawa T, et al. Transcriptional expression of survivin and its splice variants in bone marrow cells from patients with acute lymphocytic leukemia and chronic lymphocytic leukemia. Leuk Res 2003;28:487–94.
62. Caldas H, Honseley LE, Altura RA. Survivin 2a: a novel survivin splice variant expressed in human malignancies. Mol Cancer 2005;4:111.
63. Bedran A, Yoshida A, Ishikawa K, et al. Identification of a novel splice variant of the human anti-apoptotic gene survivin. Biochem Biophys Res Commun 2004;314:902–7.
64. Papapetropoulos A, Fulton D, Mahboubi K, et al. Angiopoietin-1 inhibits endothelial cell apoptosis via the Akt/survivin pathway. J Biol Chem 2002;277:9102–5.
65. Zhu L, Fukuda S, Cordis G, et al. Anti-apoptotic protein survivin plays a significant role in tubular morphogenesis of human coronary arterial endothelial cells by hypoxic preconditioning. FEBBS Lett 2001;508:369–74.
66. Ohashi H, Takagi H, Oh H, et al. Phosphatidylinositol 3-kinase/Akt regulates angiotensin II-induced inhibition of apoptosis in microvascular endothelial cells by governing survivin expression and suppression of caspase-3 activity. Circ Res 2004;94:785–93.
67. Daly C, von Wang¨, Burova E, et al. Angiopoietin-1 modulates endothelial cell function and gene expression via the transcription factor FKHR (FOXO1). Genes Dev 2004;18:1060–71.
68. Mahboubi K, Li F, Pliesca J, et al. Interleukin-11 up-regulates survivin expression in endothelial cells through a signal transducer and activator of transcription-3 pathway. Lab Invest 2001;81:327–34.
69. Kikirk-Smith NC, Mahboubi K, Pliesca J, et al. IL-11 protects human microvascular endothelium from alloinjury in vivo by induction of survivin expression. J Immunol 2004;172:1391–6.
70. Conway EM, Zwarts F, Van EV, et al. Survivin-dependent angiogenesis in ischemic brain: molecular mechanisms of hypoxia-induced up-regulation. Am J Pathol 2003;163:935–46.
71. Yang L, Cao Z, Li F, et al. Tumor-specific gene expression using the survivin promoter is further increased by hypoxia. Gene Ther 2004;11:1215–23.
72. McMurphy MS, Archer SL, Altieri DC, et al. Gene therapy targeting survivin selectively induces pulmonary vascular apoptosis and reverses pulmonary arterial hypertension. J Clin Invest 2005;115:1479–91.
73. Kornacker M, Veneris MR, Kornacker B, et al. Survivin expression correlates with apoptosis resistance after overexpression of TP53 in thymocytes enhances cell proliferation. Mol Immunol 2002;39:289–98.
74. Coumou X, Li W, Wang RH, Deng C. Inducible suppression of Fgf2 and survivin in ES cells using a combination of the RNA interference (RNAi) and the Cre-LoxP system. Nucleic Acids Res 2004;32:e85.
75. Lu GP, Cao TJ, Zhang ZY, Liu W. Multiple gene differential expression patterns in human ischemic liver; safe limit of warm ischemic time. World J Gastroenterol 2004;10:2130–3.
76. Deguchi M, Shiraki K, Inoue H, et al. Expression of survivin during liver regeneration. Biochem Biophys Res Commun 2002;297:59–64.
77. Conway EM, Pollefeyt S, Steiner-Mosonyi M, et al. Deficiency of survivin in transgenic mice exacerbates Fas-induced apoptosis via mitochondrial pathways. Gastroenterology 2002;123:819–31.
78. Sasaki T, Lopes MB, Hankins GR, Helm GA. Expression of survivin, an inhibitor of apoptosis protein, in tumors of the nervous system. Acta Neuropathol (Berl) 2002;104:105–9.
79. Jiang Y, de Bruin A, Caldas H, et al. Essential role for survivin in early brain development. J Neurosci 2005;25:6862–70.
80. Chioiu SK, Moon WS, Jones MK, Tarnawski AS. Survivin expression in the stomach: implications for mucosal integrity and protection. Biochem Biophys Res Commun 2003;309:374–9.
81. Vetter CS, Muller-Blech K, Schrama D, et al. Cytoplasmic and nuclear expression of survivin in melanocytic skin lesions. Arch Dermatol Res 2005;297:26–30.
82. Chiordino C, Cesinamo AR, Ottani D, et al. Communication: expression of the novel inhibitor of apoptosis survivin in normal and neoplastic skin. J Invest Dermatol 1999;113:415–8.
83. Wang Y, Suominen JS, Hakovirta H, et al. Survivin expression in rat testis is up-regulated by stem-cell factor. Mol Cell Endocrinol 2004;218:165–74.
84. Kumazawa Y, Kawamura K, Sato T, et al. HCG up-regulates survivin mRNA in human granulosa cells. Mol Hum Reprod 2005;11:161–6.
85. Li F. Survivin study: what is the next wave? J Cell Physiol 2003;197:8–29.
86. Wang Z, Sampath J, Fukuda S, Pelus LM. Disruption of the inhibitor of apoptosis protein, survivin, sensitizes BoA-positive cells to STIS171-induced apoptosis. Cancer Res 2005;65:8224–32.
87. Sommer KW, Schambroch CJ, Schmidt GE, et al. Inhibitor of apoptosis protein (IAP) survivin is up-regulated by oncogenic c-H-Ras. Oncogene 2003;22:4266–80.
88. Fulda S, Debatin KM. Sensitization for anticancer drug-induced apoptosis by the chemopreventive agent resveratrol. Oncogene 2004;23:6702–11.
89. buEl-Asrar AM, Dralands L, Missotten L, et al. Expression of apoptosis markers in the retinas of human subjects with diabetes. Invest Ophthalmol Vis Sci 2004;45:2780–6.
90. Aoki Y, Feldman GM, Tosato G. Inhibition of STATA3 signaling induces apoptosis and decreases survivin expression in primary effusion lymphoma. Blood 2003;101:1535–42.
91. Hoffman WH, Bade S, Ziflove JT, et al. Transcriptional repression of the anti-apoptotic survivin gene by wild type p53. J Biol Chem 2002;277:3247–57.
92. Mirza A, McGuirk M, Hockenberry TN, et al. Human survivin is
negatively regulated by wild-type p53 and participates in p53-dependent apoptotic pathway. Oncogene 2002;21:2613–22.

94. Jiang Y, Saavedra HI, Holloway MP, et al. Aberrant regulation of survivin by the RB/E2F family of proteins. J Biol Chem 2004;278:40511–20.

95. Tracey L, Perez-Rosado A, Artiga MJ, et al. Expression of the NF-κB targets BCL2 and BIRC5/survivin characterizes small B-cell and aggressive B-cell lymphomas, respectively. J Pathol 2005;206:123–34.

96. Xu Y, Fang F, Ludewig G, et al. A mutation found in the promoter region of the human survivin gene is correlated to overexpression of survivin in cancer cells. DNA Cell Biol 2004;23:527–37.

97. Carter BZ, Milella M, Altieri DC, Andreff M. Cytokine-regulated expression of survivin in myeloid leukemia. Blood 2001;87:2784–90.

98. Fortugno P, Beltrami E, Pescia J, et al. Regulation of survivin function by Hsp90. Proc Natl Acad Sci U S A 2003;100:13791–6.

99. Song Z, Yao X, Wu M. Direct interaction between survivin and Smac/DIABLO is essential for the anti-apoptotic activity of survivin during taxol-induced apoptosis. J Biol Chem 2003;278:23130–40.

100. Wang Z, Fukuda S, Pelus LM. Survivin regulates the p53 tumor suppressor gene family. Oncogene 2004;23:8146–53.

101. Dohi T, Beltrami E, Wall NR, et al. Mitochondrial survivin inhibits apoptosis and promotes tumorigenesis. J Clin Invest 2004;114:1117–27.

102. Sarela AI, Verbeke CS, Ramsdale J, et al. Expression of the antisense gene, survivin, predicts death from recurrent colorectal cancer. Gut 2000;46:645–50.

103. Osawa M, Nakamura K, Nishi N, et al. In vivo self-renewal of c-KIT+ Sca-1+ Lin(low−) hematopoietic stem cells. J Immunol 1996;156:3207–14.

104. Osawa M, Hanada K, Hamada H, Nakachi H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. Science 1996;272:242–5.

105. Nibley WE, Spangrude GJ. Primitive stem cells alone mediate rapid marrow recovery and multilineage engraftment after transplantation. Bone Marrow Transplant 1998;21:345–54.

106. Zijlmans JM, Visser JW, Lateveer L, et al. The early phase of engraftment after murine blood cell transplantation is mediated by hematopoietic stem cells. Proc Natl Acad Sci U S A 1998;95:725–9.

107. Kato J, Kuwabara Y, Mitani M, et al. Expression of survivin in esophageal cancer: correlation with the prognosis and response to chemotherapy. Int J Cancer 2001;95:92–5.

108. Ikeguchi M, Kaibara N. Survivin messenger RNA expression is a good prognostic biomarker for oesophageal carcinoma. Br J Cancer 2002;87:883–7.

109. Grabowski P, Kuhniet T, Muhr-Wilkenshoff F, et al. Prognostic value of nuclear survivin expression in oesophageal squamous cell carcinoma. Br J Cancer 2003;88:115–9.

110. Lu B, Gonzalez A, Massion PP, et al. Nuclear survivin as a biomarker for non-small-cell lung cancer. Br J Cancer 2004;91:537–40.

111. Monzo M, Rosell R, Felip E, et al. Expression of the antisense gene, survivin, in non-small-cell lung cancers. J Clin Oncol 1999;17:2100–4.

112. Kren L, Brazdil J, Hermanova M, et al. Prognostic significance of expression of survivin messenger RNA as a prognosis marker in non-small-cell lung cancer. Jpn J Clin Oncol 2003;33:194–201.

113. Falleni M, Pellegrini C, Marchetti A, et al. Survivin gene expression in early-stage non-small cell lung cancer. J Pathol 2003;200:620–6.

114. Ferrandina G, Legge F, Martinelli E, et al. Survivin expression in ovarian cancer and its correlation with clinico-pathologic, surgical and apoptosis-related parameters. Br J Cancer 2005;92:297–71.

115. Cohen C, Lohmann CM, Cotsonis G, et al. Survivin expression in ovarian carcinoma: correlation with apoptotic markers and prognosis. Mol Pathol 2003;16:574–83.

116. Fungusaro JR, Jiang Y, Holloway MP, et al. Survivin, survivin-2B, and survivin-ιε3 expression in medulloblastoma: biologic markers of tumour morphology and clinical outcome. Br J Cancer 2005;92:359–65.

117. Chakravarti A, Noll E, Black PM, et al. Quantitatively determined survivin expression levels are of prognostic value in human gliomas. J Clin Oncol 2002;20:1063–8.

118. Kajiwara Y, Yamaski F, Hama S, et al. Expression of survivin in astrocytic tumors: correlation with malignant grade and prognosis. Cancer 2003;97:1077–83.

119. Knutsen A, Adell G, Sun XF. Survivin expression is an independent prognostic factor in rectal cancer patients with and without preoperative radiotherapy. Int J Radiat Oncol Biol Phys 2004;60:149–55.

120. Sarela AI, Scott N, Ramsdale J, et al. Immunohistochemical detection of the anti-apoptosis protein, survivin, predicts survival after curative resection of stage II colorectal carcinomas. Ann Surg Oncol 2001;8:305–10.

121. Komuro Y, Watanabe T, Tsurita G, et al. Survivin expression in ovarian carcinoma: correlation with apoptotic markers and prognosis. Mod Pathol 2004;17:264.

122. Grossman D, McNiff JM, Li F, Altieri DC. Expression and targeting of the apoptosis inhibitor, survivin, in human melanoma. J Invest Dermatol 1999;113:1076–81.

123. Wurl P, Kaplan M, Meye A, et al. Co-expression of survivin and TERT and risk of tumour-related death in patients with soft-tissue sarcoma. Lancet 2002;359:943–5.

124. Kaplan M, Kotschz B, Bartel F, et al. Elevated expression level of survivin protein in soft-tissue sarcomas is a strong independent predictor of survival. Clin Cancer Res 2003;9:1098–104.

125. Kaplan M, Kohler T, Kampf C, et al. Increased survivin transcript levels: an independent negative predictor of survival in soft tissue sarcoma patients. Int J Cancer 2001;95:360–3.

126. Sarela AI, Verbeke CS, Ramsdale J, et al. Expression of survivin, a novel inhibitor of apoptosis and cell cycle regulatory protein, in pancreatic adenocarcinoma. Br J Cancer 2002;86:886–92.

127. Yoshida H, Sumi T, Huy N, et al. Expression of survivin and matrix metalloproteinases in adenocarcinoma and squamous cell carcinoma of the uterine cervix. Oncol Rep 2003;10:45–9.

128. Lee JP, Chang KH, Han JH, Ryu HS. Survivin, a novel anti-apoptosis inhibitor, expression in uterine cervical cancer and relationship with prognostic factors. Int J Gynecol Cancer 2005;15:113–9.

129. Ikeguchi M, Ueda T, Sakatani T, et al. Expression of survivin messenger RNA correlates with poor prognosis in patients with hepatocellular carcinoma. Diagn Mol Pathol 2002;11:33–40.

130. Tammi I, Richter S, Oltersdorf D, et al. High expression levels of X-linked inhibitor of apoptosis protein and survivin correlate with poor overall survival in childhood de novo acute myeloid leukemia. Clin Cancer Res 2004;10:3737–44.

131. Carter BZ, Kornblau SM, Tsao T, et al. Caspase-independent cell death in AML: caspase inhibition in vitro with pan-caspase inhibitors or in vivo by XIAP or survivin does not affect cell survival or prognosis. Blood 2003;102:4179–86.

132. Chen J, Wu W, Tahir SK, et al. Down-regulation of survivin by antisense oligonucleotides increases apoptosis, inhibits cytokinesis and anchorage-independent growth. Neoplasia 2000;2:235–41.

133. Schlette EJ, Medeiros LJ, Goy A, et al. Survivin expression predicts poorer prognosis in anaplastic large-cell lymphoma. J Clin Oncol 2004;22:1682–8.

134. Adida C, Haïoun C, Gauvard P, et al. Prognostic significance of survivin expression in diffuse large B-cell lymphomas. Blood 2000;96:1921–5.

135. Geddis AE, Kaushansky K. Megakaryocyte expression in vivo of survivin, an inhibitor of apoptosis, in human melanoma. J Invest Dermatol 2002;118:376–80.

136. Frost M, Jarboe EA, Orlicky D, et al. Immunohistochemical localization of survivin in benign cervical mucosa, cervical dysplasia, and invasive squamous cell carcinoma. Am J Clin Pathol 2002;117:738–44.

137. Weikert S, Schrader M, Krause H, et al. Inhibitor of apoptosis protein (IAP) survivin is expressed in human testicular germ cell tumors and normal testes. Cancer Lett 2005;223:331–7.

138. Nasu S, Yagihashi A, Izawa A, et al. Survivin mRNA expression in patients with breast cancer. Anticancer Res 2002;22:1839–43.