Is Survivin the Potential Achilles’ Heel of Cancer?

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Survivin, the smallest member of the inhibitors of apoptosis proteins (IAPs), plays an important role in the control of apoptosis, cell division, and cell migration/metastasis. Survivin is expressed and required for normal fetal development but is then generally no longer present in most adult tissues. However, reexpression of survivin is observed in numerous human cancers where presence of the protein is associated with enhanced proliferation, metastasis, poor prognosis, and decreased patient survival. Given the relatively selective expression in cancer cells, but not in normal tissue (tumor-associated antigen), and its importance in tumor cell
biology, survivin has emerged as an attractive target for cancer treatment. Here, we discuss some aspects of survivin biology by focusing on why the protein appears to be so important for cancer cells and then discuss strategies that harness this dependence to eradicate tumors and situate survivin as a potential Achilles’ heel of cancer. © 2011 Elsevier Inc.

I. INTRODUCTION

A. Survivin, A Member of the Inhibitors of Apoptosis Protein Family

The inhibitors of apoptosis proteins (IAPs) were initially described by Miller’s group as proteins whose function related to the regulation of apoptosis (Crook et al., 1993; Duckett et al., 1996; LaCasse et al., 1998; Uren et al., 1996). Since then a number of additional roles have emerged, including regulation of chromosome segregation during mitosis, cooper metabolism, and cell signaling. IAPs are generally multimodular proteins characterized by the presence of a variable number of baculovirus inhibitory repeat (BIR) domains in combination with ubiquitin-ligase RING domains and caspase-associated recruitment domains (CARD). The combination of elements is thought to define the properties of individual IAPs. The BIR domain represents the hallmark motif of this family of proteins and the only element common to all IAPs. This 70 amino acid module that coordinates zinc between cysteine and histidine residues mediates protein–protein interactions between different IAPs, as well as with other proteins. In some IAPs, only a single BIR is present (such as survivin, ILP2, and BRUCE) while others contain three such elements in tandem (XIAP, c-IAP1, cIAP2, and NAIP) (Srinivasula and Ashwell, 2008).

The most particular and smallest member of IAP family is survivin, a 16-kDa protein with only a single NH2-terminal BIR domain and a COOH-terminal coiled-coil region for microtubule interaction (Altieri, 2008). As other IAPs, survivin has been associated with the control of numerous cellular functions, such as inhibition of apoptosis, cell cycle progression, control of spindle formation and kinetochore attachment, angiogenesis, and stress responses (Altieri, 2006b). Since expression of survivin is elevated in human tumors but mostly absent in normal tissue and its presence in tumors is associated with poor prognosis, survivin has attracted the attention of a large number of researchers working on different aspects of cancer. This review will focus on the discussion of strategies to target survivin as potential avenues for the development of novel cancer therapies.

Survivin is encoded by a complex gene (*BIRC5*) located on the human 17q25 chromosome that contains four well-defined and three hidden exons (for more details concerning survivin isoforms, see Li (2005); Li
Alternative splicing of pre-mRNA generates four splice variants designated survivin 2α, survivin 2B, survivin Δ3Ex, and survivin 3B (Table I). Of these spliced isoforms, only survivin 3B contains an intact BIR domain (Li and Ling, 2006). Survivin wt levels are generally elevated in proliferating cells and particularly in cancer cells. By comparison, survivin 2α, survivin 2B, and survivin Δ3Ex levels are extremely low and, for that reason, are often not considered particularly relevant, although some specific functions have been ascribed to individual variants (Table I) (Caldas et al., 2005a; Noton et al., 2006). The survivin isoforms 2α, 2B, and 3B are predominantly cytosolic during interphase and fail to localize to the midbodies, as does survivin wt in cytokinesis (Caldas et al., 2005b; Knauer et al., 2007; Mahotka et al., 2002), suggesting that these survivin splice variants do not participate in cell division (Noton et al., 2006). Interactions between different survivin isoforms are likely to exist, as suggested by coimmunoprecipitation of survivin Δ3Ex with survivin wt. Complexes containing these two variants localize to mitochondria and have been ascribed antiapoptotic functions (Caldas et al., 2005b). Alternatively, survivin 2α and 2B have been suggested to promote apoptosis (Caldas et al., 2005a; Zhu et al., 2004). Also, survivin Δ3Ex is suggested to promote angiogenesis in endothelial cells in vivo (Caldas et al., 2007). However, it is currently not well understood how survivin isoforms exert their functions and what protein-binding partners may be required. For this reason, the following discussion will focus exclusively on wild-type survivin.

II. CELL BIOLOGY OF SURVIVIN

A. Regulation of Survivin Expression

Survivin wt (hereafter referred to as survivin) is abundantly expressed during development and in some adult tissues, such as thymus, placenta (Ambrosini et al., 1997), liver, and arterial muscle (Fukuda and Pelus,
More recently survivin was also shown to be abundantly expressed in the human stomach epithelium (Valenzuela et al., 2010). However, the level of expression of survivin is generally lower in normal tissues in comparison to tumors (Fukuda and Pelus, 2006). Survivin expression is extensively regulated by transcriptional, as well posttranscriptional and posttranslational mechanisms in a cell cycle dependent manner (Fig. 1). At the posttranslational level, it has been suggested that phosphorylation and ubiquitination are important for survivin functions and for the control of survivin protein levels. In this way, survivin phosphorylation by aurora kinase B and the polo-like kinase-1 are crucial for survivin localization to the centrosome and for chromosome alignment respectively, and both are important during cell division (Colnaghi and Wheatley, 2010; Wheatley et al., 2004, 2007)

Survivin phosphorylation also is involved in protection against cell death. Phosphorylation by p34cdc/cyclin B protects the cells from

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**Fig. 1**  Survivin regulation. Survivin is regulated at the transcriptional level by transcription factors that either favor or suppress expression. The factors β-catenin/TCF-Lef, HIF1, Sp1, and Stat 3 induce while the tumor suppressor genes p53, Rb, and PTEN repress survivin expression. At the posttranslational level, survivin is phosphorylated by Polo Like kinase 1, Aurora B kinase, and p34cdc2/cyclin B. These events enhance protein stability, promote mitosis, and protect against cell death. Furthermore, survivin is monoubiquitinated and targeted for subsequent degradation via the proteasome pathway by as yet to be identified ligases.
induction of apoptosis during cell cycle progression as a consequence of the interaction between survivin and caspase-9 in midbodies of dividing cells (O’Connor et al., 2000a). Additionally, augmented p34cdc kinase activity results in increased survivin expression and cell viability (O’Connor et al., 2002). Conversely, the downregulation of cyclin B leads to a reduction in survivin protein levels and sensitizes cells to TRAIL (Kim et al., 2010). Altogether, these observations strongly suggest that survivin phosphorylation is an important mechanism by which cancer cells may avoid cell death.

In addition, survivin is ubiquitinated in a highly dynamic process, which involves nondegradative and degradative cycles. Nondegradative cycles of survivin ubiquitination/deubiquitination are proposed to be important in mitotic control of chromosome alignment and degradative cycles are responsible for the rapid decline in survivin protein levels in the G1 phase (Vong et al., 2005; Zhao et al., 2000). The control of protein levels by phosphorylation and ubiquitination, suggest the existence of multiple fine tuning mechanisms to control survivin protein levels in normal cells. However, it is not yet understood how dysregulation of these mechanisms can contribute to constitutive expression of survivin observed in cancer cells.

On the other hand, survivin is highly regulated at the transcriptional level by several transcriptional factors. For example, transcriptional factors such as β-catenin-TCF/LEF (Kim et al., 2003; Zhang et al., 2001), Sp1 (Li and Altieri, 1999), Stat3 (Aoki et al., 2003), and HIF1α (Peng et al., 2006; Yang et al., 2004) promote survivin mRNA and protein expression. In addition, survivin is strongly repressed by p53 (Hoffman et al., 2002; Mirza et al., 2002; Xia and Altieri, 2006), Rb (Jiang et al., 2004), by the dual-phosphatase PTEN (Guha et al., 2009), and the scaffolding protein caveolin-1 (Torres et al., 2006, 2007). Despite the existence of such intricate and extensive control mechanisms, cancer cells suffer alterations that lead to constitutive expression of survivin. For example, gain of function in the β-catenin TCF/LEF pathway (Daidone et al., 2004; Kim et al., 2003; Zhang et al., 2001), activation of H-Ras, Bcr-Abl (Sommer et al., 2003; Wang et al., 2005), and inactivation of p53, Rb, and PTEN (Cully et al., 2006; Knudsen and Knudsen, 2008; Levine and Oren, 2009) or loss of caveolin-1 expression (Quest et al., 2004, 2008) are events associated with the development of cancer that contribute to augmented expression of survivin.

**B. The Multiple Functions of Survivin**

Survivin regulates important pathways implicated in cellular homeostasis, including cell cycle progression, cell proliferation, and cell death. This
protein is essential for several stages of cell division and associates physically with polymerized microtubules, mitotic chromatin-associated spindle microtubules, as well as the kinetochore-associated chromosomal passenger complex, thereby regulating progression through mitosis at different checkpoints (Ambrosini et al., 1997; Deveraux and Reed, 1999; Li et al., 1998). The chromosomal passenger protein complex (CPC) is important in cell division, due to its role in surveillance of early and late mitotic events, such as proper chromosome alignment. In the CPC, survivin together with Borealin, controls CPC localization throughout cell division, probably in a BIR domain-dependent manner (Lens et al., 2006; Vader et al., 2006). Moreover, a non-CPC pool of survivin is involved in mitosis, via modulation of microtubule dynamics at the kinetochore and also by promoting spindle formation (Altieri, 2006a). Finally, recent findings show that survivin interacts with phosphorylated histone, thereby connecting the CPC with chromatin (Kelly et al., 2010). In this manner, survivin plays a cooperative role by regulating progression of mitosis. Thus, inhibition of survivin expression with antisense oligonucleotide treatment or chemotherapeutic drugs has been associated with defects in cell division, characterized by polyploidy accompanied by the formation of multinucleated cells, increases in centrosome numbers, and mitotic catastrophe (Li et al., 1999; Ngan et al., 2008), a type of cell death triggered due to failure in mitosis or DNA damage (Castedo et al., 2004).

In addition to its role in facilitating cell cycle progression, there is a considerable body of evidence indicating that the presence of survivin in tumor cells is essential for avoiding apoptotic cell death. With the exception of X-linked IAP protein (XIAP), the rest of the IAP family members are generally thought to exert their antiapoptotic function via mechanisms that do not involve direct caspase inhibition (Eckelman et al., 2006). Despite, this advance, the precise mechanisms by which survivin prevents cell death remain heavily debated. Survivin antiapoptotic function seems to rely on its interactions with other molecules such as hepatitis B X-interacting protein (HBXIP) (Marusawa et al., 2003), SMAC/DIABLO (Song et al., 2003), XIAP (Dohi et al., 2007), its mitochondrial localization (Dohi et al., 2004), or even extracellular presence on exosomes (Khan et al., 2009, 2011). Mitochondrial survivin has been proposed to control the release of SMAC/DIABLO from mitochondria induced by chemotherapeutic drugs and thereby reduce the apoptotic cell index (Ceballos-Cancino et al., 2007; Dohi et al., 2004). Thus, rather than directly inhibiting effector caspases, survivin interferes with upstream events, such as SMAC/DIABLO release, associated with initiation of the intrinsic (mitochondrial) pathway of apoptosis. Moreover, survivin has been associated with the inhibition of release of the apoptosis-inducing factor (AIF) from mitochondria (Liu et al., 2004) and survivin silencing is
associated with AIF nuclear-translocation (Croci et al., 2008). Taken together, these results suggest that survivin expression is important in the prevention of caspase-dependent and independent apoptosis.

A novel function proposed for survivin involves the promotion of cell migration and metastasis. Two different lines of evidence support this role. The first one is related to the identification of an extracellular pool of survivin associated with peripheral blood leukocytes and cancer cells (Bokarewa et al., 2005; Khan et al., 2009, 2011; Mera et al., 2008). In the latter case, extracellular survivin enhances proliferation, colony formation, migration, and resistance to chemotherapeutic drugs, as well as UV radiation induced cell death (Khan et al., 2009, 2011). The second line of evidence suggests the existence of a survivin–XIAP protein complex that promotes cell migration in vitro in an NF-κB dependent manner and metastasis in vivo, in a fashion that is independent of survivin’s role in apoptosis (Mehrotra et al., 2010). Additionally, survivin also favors melanoma cell mobility by a mechanism involving Akt activation and α5 integrin upregulation (McKenzie et al., 2010). Currently, it is not clear how survivin acts in an autocrine–paracrine fashion, while an understanding of how survivin promotes cell migration and metastasis is becoming more tangible.

Apoptosis, or programmed cell death, is a widely employed mechanism that permits removal of unneeded, damaged, or potentially harmful cells. As such, apoptosis is a stringently regulated process that is critical for normal embryonic development and homeostasis in adult tissues (Thornberry and Lazebnik, 1998). Dysregulation of this process and increased resistance to cell death, a common feature of malignant cells (Hanahan and Weinberg, 2000; Reed, 1999), represents a significant obstacle to successful therapy of human cancer (Rudin and Thompson, 1997). As outlined above, survivin expression is strongly and globally deregulated in transformed human cells of different origins, leading to overexpression in all phases of the cell cycle, not just during the G2–M phase. Such aberrant expression is associated with enhanced tumor cell viability. Presence of survivin in tumors is also linked to poor responsiveness to chemotherapy (Koh, 1991), increased tumor aggressiveness, enhanced metastasis (Glinsky et al., 1997), and decreased patient survival (Adida et al., 1998; Kawasaki et al., 1998; Monzo et al., 1999; Swana et al., 1999; Tanaka et al., 2000).

C. Molecular Targeting of Survivin in Cancer Therapy

Survivin upregulation in cancer cells promotes resistance to cancer therapies and transformed cells are exquisitely sensitive to reduction of survivin levels or function, resulting in dysregulation of mitotic progression (Li
et al., 1999; Muchmore et al., 2000) and spontaneous apoptosis (Grossman et al., 1999; Olie et al., 2000). Consequently, several approaches have been developed to target survivin expression or function in cancer cells with the objective of impairing tumor growth and/or sensitizing tumors to chemotherapeutic drugs (Fig. 2). Proof of concept was provided early on in nontransformed cells, where antisense

![Diagram of survivin-based cancer therapies](image)

**Fig. 2** Survivin-based cancer therapies. Survivin expression in tumor cells can be downregulated by small molecule inhibitors (e.g., YM155) that suppress transcriptional activity or by agents that promote the degradation of survivin mRNA and/or block protein translation, such as antisense-, ribozyme-, and RNAi-based molecules. Other strategies that target survivin at the protein level by interfering with its multiple functions and/or promoting its degradation include dominant negative mutants (e.g., C84A, T34A) and peptide antagonists (e.g., shepherdin). Tumor cells presenting survivin-derived peptides in complexes with MHC class I molecules at the cell surface are targets of survivin-specific cytotoxic CD8+ T cells that can be induced by immunotherapeutic strategies, such as dendritic cell (DC)-, peptide-, and DNA-based vaccines.
oligonucleotide-mediated downregulation of survivin was shown to enhance caspase-3 activity, spontaneous apoptosis, and aberrant mitosis, while interference with survivin homologs in Caenorhabditis elegans and yeast lead to lethal defects in cytokinesis (Fraser et al., 1999; Li et al., 1998, 1999; Uren et al., 1999). Also, survivin antisense molecules were shown to mediate spontaneous apoptosis in human melanoma cells (Grossman et al., 1999). Several studies have consistently demonstrated the efficacy of this strategy in inducing apoptosis or enhancing the susceptibility to chemo- and radiotherapy of tumor cells from different tissues (Cao et al., 2004; Carter et al., 2006; Hansen et al., 2008; Lu et al., 2004). Alternative approaches employed to downregulate survivin expression, including ribozymes (Pennati et al., 2002, 2003, 2004) and RNA interference (Carvalho et al., 2003; Izquierdo, 2005), have yielded similar results. Importantly, all three strategies have been shown to be effective in vivo using mouse xenograft models. Antisense oligonucleotides enhanced the sensitivity of lung cancer tumors to radiotherapy (Cao et al., 2004) and inhibited the development and growth of a non-Hodgkin’s lymphoma model. Human prostate cancer cells expressing a survivin-targeting ribozyme formed tumor nodules but were unable to progress and the tumors completely regressed within a few weeks (Pennati et al., 2004). Likewise, knock down of survivin expression by short hairpin RNA-encoded vectors decreased growth of oral squamous cell carcinoma (Jiang et al., 2006), as well as breast and cervical tumors (Li et al., 2006).

Given the success in such preclinical experimental settings, survivin antagonists are now being tested in the clinic. The survivin-targeting antisense molecule LY2181308 has been tested in phase I trials and was shown to be well tolerated in patients with different solid tumors (Talbot et al., 2010; Tanioka et al., 2010). Importantly, at doses that had an acceptable toxicity profile, LY2181308 was shown to accumulate in tumor tissues as determined by immunohistochemistry and ELISA analysis (Talbot et al., 2010). Consistent with the accumulation of LY2181308 in tumors, a significant reduction in survivin mRNA and protein levels, as well as enhanced expression of apoptotic markers was detected in post-treatment tumor biopsies. Despite these encouraging findings, no objective clinical responses were observed. Therefore, it would be interesting to determine in future studies whether the combination of LY2181308 and other proapoptotic agents might result in enhanced antitumor effects (trial identification codes: NCT00620321, NCT01107444, NCT00642018).

Small-molecule inhibitors that decrease survivin promoter activity have also been used to target survivin gene expression. YM155, an imidazolium-based compound, was identified in a screen using a luciferase gene promoter activity assay and found to accumulate into human prostate tumor xenografts causing dose-dependent tumor suppression
that was paralleled by decreased intratumoral survivin expression (Nakahara et al., 2007). YM155 also showed enhanced antitumor effects when combined with chemo- or radiotherapy in a xenograft model of human nonsmall cell lung cancer (NSCLC) (Iwasa et al., 2008, 2010). In phase I clinical studies, YM155 was safely administered and elicited moderate antitumor activity in patients with advanced solid cancers and lymphoma (Satoh et al., 2009; Tolcher et al., 2008). In one trial involving 41 patients, 3 out of 5 patients with non-Hodgkin’s lymphoma showed clinical responses, with 2 being partial and 1 a complete responder. Furthermore, two of nine patients with hormone-refractory, docetaxel-treated prostate cancer, prostate-specific antigen levels declined although later the disease progressed again (Tolcher et al., 2008). In another phase I trial, YM155 administration stabilized disease progression in 9 out of 34 patients (Satoh et al., 2009). Modest results were obtained using this drug as a single agent in phase II clinical trials including patients with advanced NSCLC (Giaccone et al., 2009) or unresectable stage III or IV melanoma (Lewis et al., 2011). Several trials assessing the safety and efficacy of using YM155 in association with chemotherapeutic drugs are now underway (trial identification codes: NCT00514267, NCT01100931, NCT01007292, NCT01038804, NCT01009775).

Other strategies have been pursued that target survivin at the protein level by interfering with its multiple functions. For instance, expression of a dominant negative survivin variant, with a cysteine 84 to alanine (C84A) substitution in the baculovirus IAP repeat motif that prevents dimerization, triggers apoptosis, and mitotic catastrophe in melanoma (Grossman et al., 1999), gastric (Tu et al., 2003), and colon (Tu et al., 2005) cancer cells in vitro, as well as inhibits tumorigenesis and angiogenesis in vivo (Tu et al., 2003, 2005). Similarly, the nonphosphorylatable T34A survivin mutant has been shown to act as a dominant negative variant that promotes spontaneous and drug-induced apoptosis, prevents tumor formation, and drastically reduces the growth of established human melanoma (Grossman et al., 2001b) and breast cancer (Mesri et al., 2001b) tumors in xenograft models. Intratumoral application of this survivin mutant, using an adenovirus infection protocol, also reduced tumor-associated blood vessel growth by enhancing endothelial cell apoptosis in vivo (Mesri et al., 2001a). Moreover, pharmacological inhibition of Thr34-phosphorylation has been shown to be effective in suppressing the growth of breast cancer xenografts when combined with anticancer agents, such as taxol (O’Connor et al., 2002) and adriamycin (Wall et al., 2003). More recently, a cell-permeable version of a survivin-derived peptide (Lys79–Leu87) that disrupts survivin-heat shock protein 90 (Hsp90) interaction was designed to inhibit Hsp90 chaperone function by
competing with its ATP-binding pocket and destabilizing several Hsp90 client proteins. This peptide, called shepherdin, induced massive death of tumor cells of different origin by both apoptotic and nonapoptotic mechanisms without affecting normal cells (Plescia et al., 2005). Systemic administration of shepherdin reduced survivin expression and was not toxic but inhibited the growth of human prostate and breast tumors in vivo.

Taken together, these data strongly indicate that functions of survivin linked to regulation of apoptosis and cell cycle progression are important for tumor survival, a point that may explain the prevalence of this protein in almost all human tumors studied. Hence, successful manipulation of the survivin pathway either alone or in combination with chemotherapy can be expected to be therapeutically useful in the treatment of human tumors. Moreover, since survivin meets all the requirements of a tumor-associated antigen (see below), immune-based strategies are being pursued as a novel, exciting, and promising area of development in the struggle against cancer. Indeed, currently phase I/II trails are underway that seek to exploit immune-targeting of survivin to treat cancer. Given their emerging potential, the rest of this review will focus the discussion on survivin as a tumor-associated antigen and a target for such immune-based strategies.

III. SURVIVIN AS A GENERIC TUMOR-ASSOCIATED ANTIGEN

Widespread expression in several types of human cancers, general absence in the respective normal adult tissues and a requirement for tumor cell survival identify survivin as an almost ideal “universal” tumor-associated antigen (TAA). Survivin is overexpressed in most human cancers (Ambrosini et al., 1997; Velculescu et al., 1999), including lung, colon, breast, pancreas, stomach, liver, ovary, and prostate cancer, as well as in melanoma and hematopoietic malignancies. The survivin transcript was identified as one of the top four genes invariably upregulated in different types of human tumors, but absent in normal cells of the same tissue (Velculescu et al., 1999). Interestingly, survivin is not only overexpressed in malignant cells but also in the tumor-associated stroma (Kawasaki et al., 2001). Survivin is a growth factor-inducible gene that is strongly upregulated in actively dividing endothelial cells forming blood vessels where it plays a prominent role in counteracting apoptotic stimuli and stabilizing the vascular network (Mesri et al., 2001a; O’Connor et al., 2000b; Papapetropoulos et al., 2000; Tran et al., 1999). Consequently, different approaches that target survivin effectively
suppress tumor-associated angiogenesis in vitro and in vivo (Blanc-Brude et al., 2003; Coma et al., 2004).

A. Immune Recognition of Survivin

The immune system can sense malignant transformation of cells and generate adaptive immune responses against novel antigens (e.g., mutated or abnormally modified proteins), as well as increased levels of preexisting antigens (e.g., overexpressed antigens) (Castelli et al., 2000). The intracellular localization of survivin makes it a suitable target for adaptive T cell-mediated immunity (Fig. 2). T cells recognize unique antigen-derived epitopes bound to MHC molecules through the T-cell receptor (TCR). The typical antigen-derived epitope is a short peptide, between 8 and 20 amino acids in length, which is obtained by the degradation of protein antigens in the cytosol. These peptides are transported into the ER where they associate with MHC molecules and then are transported to the cell surface (Pamer and Cresswell, 1998). Cytotoxic CD8+ T lymphocytes (CTL) recognize such peptides bound to MHC class I molecules (signal 1) and after appropriate coreceptor (signal 2) and cytokine (signal 3) stimulation acquire the cytotoxic effector phenotype that enables them to systemically kill cells presenting the respective epitope/MHC I complex. Helper CD4+ T cells recognize antigen-derived peptides complexed with MHC class II molecules and the effector phenotype is largely based on the secretion of diverse cytokines that sustain and regulate the CTL and other immune responses.

Unlike pathogens, cancer cells share a vast repertoire of antigens with normal cells and TAAs that are recognized by CTLs are mainly nonmutated self-antigens. As a mechanism to avoid immune attack against self-antigens (autoimmunity), potentially self-reactive TAA-specific T cells are either eliminated or become regulatory T cells (immune tolerance). These facts are underscored by the generally low frequencies of tumor-reactive T cell precursors or the presence of tumor-reactive T cells with low TCR affinity.

Notably, spontaneous antisurvivin CD8+ T-cell responses have previously been detected in patients with breast cancer, colon cancer, lymphoma, leukemia, melanoma, and neuroblastoma (Andersen et al., 2001a,b; Casati et al., 2003; Coughlin et al., 2006; Grube et al., 2007; Reker et al., 2004a,b; Siegel et al., 2004). Survivin-reactive T cells isolated with magnetic beads coated with MHC/peptide complexes were demonstrated to have cytotoxic activity against HLA-matched tumors of different tissue origin (Andersen et al., 2001b). Additionally, survivin-specific CTLs were generated from healthy donors or patients with leukemia that could lyse a panel of survivin-expressing cell lines derived from renal cell,
breast and colon carcinomas, melanomas, and multiple myelomas, as well as primary leukemias (Schmidt et al., 2003). Following these studies, survivin-specific CTL responses restricted to several human MHC class I molecules have been detected including HLA-A1, HLA-A2, HLA-A3, HLA-A11, HLA-A24, and HLA-B35, and the cognate epitopes were identified (Reker et al., 2004a,b). Survivin CTL epitopes restricted to other haplotypes have also been suggested to exist (Bachinsky et al., 2005). Helper CD4+ T cells play a central role in orchestrating the immune response against the tumor cells, as well as having a direct role in tumor rejection. An early study showed that in addition to survivin-specific CTLs also CD4+ T cell responses against survivin could be detected in colorectal cancer patients (Casati et al., 2003). More recently, a study revealed the importance of human CD4+ T cells in the development of survivin-specific CTLs in vitro using pulsed DCs (Kim et al., 2008). Survivin-specific epitopes restricted to multiple HLA II alleles were identified in another study where CD4+ T cell responses were detected in healthy individuals, as well as in cancer patients (Wang et al., 2008). Taken together, these results validate survivin as a relevant and prominent immunogenic TAA to which the T cell repertoire has not been completely eliminated during the development of central immune tolerance.

Despite the existence of systemic TAA-specific CTL responses, tumors can progress and lead to high rates of mortality, generally associated with tumor metastasis. In addition to the presence of T cell responses, it is extremely important that tumor-reactive T cells migrate to and infiltrate the tumor site and maintain an activated effector phenotype to achieve objective clinical benefit (Nakano et al., 2001; Sato et al., 2005). Despite the detection of survivin-specific CTLs in both primary tumors and metastases from patients with melanoma and breast cancer (Andersen et al., 2001b; Reker et al., 2004a), it was shown that patients with malignant neuroblastomas can have relatively high numbers of survivin-specific T cells in the periphery, but very limited numbers in the tumor (Coughlin et al., 2006). Insufficient antitumor immunity is probably the consequence of immunosuppressive mechanisms elicited by tumors that inhibit effector function and tumor infiltration of T cells, thereby contributing to cancer progression (Mittendorf and Sharma, 2010). A major component of tumor-induced immunosuppression is the induction of regulatory T cells (Tregs), which accumulate at the tumor site and suppress the effector functions of CTLs in a cell contact-dependent fashion through mechanisms that are not fully understood (Mougiakakos et al., 2010). Recently, circulating survivin-specific Tregs displaying potent suppressive functions, albeit at low frequency, were detected in a patient with metastatic melanoma (Vence et al., 2007). The presence of survivin-specific Tregs may represent a mechanism to avoid immune targeting of this
self-antigen and strategies that rid or block this suppressive population can potentially be used to improve survivin-specific antitumor immune responses. A caveat here, however, is that these same mechanisms might also favor the development of autoimmune diseases, as will be discussed later on (see Section IV).

### B. Experimental Vaccines Targeting Survivin

Despite important advances over the past 30 years in defining the molecular features that lead to cancer and, as a consequence, the development and the use of new therapeutic drugs, the majority of cancer patients with recurrent and advanced disease will succumb to their tumors. Immunotherapy has attracted numerous efforts to develop new strategies to combat cancer. However, it has been very difficult to translate the often-promising results obtained in vitro and in animal models into the clinic. Indeed, a very limited number of studies have shown objective clinical responses, especially when the aim was to stimulate immunity in vivo (active immunotherapy) (Rosenberg et al., 2004). The results of immunotherapy trials suggest that inducing tumor-specific T cell responses could, in some instances, cause the regression of a tumor or stabilization of the disease (Bendandi et al., 1999; Rosenberg and Dudley, 2009). However, a better understanding of the mechanisms that may explain why immunotherapy fails to control the remaining cancer cell populations needs to be developed. A common observation in individuals presenting antigen-specific immunity is the appearance of tumor cell populations that lack the targeted antigen. This immune escape mechanism of tumors could be mediated by the selective survival of those tumor cell populations that avoid T cell recognition, a phenomenon referred to as immune editing. Several TAAs are “not essential” for tumor cell survival and when CTL responses are induced by therapeutic measures, such as by vaccination, tumor cells lacking the expression of these antigens are likely to have a pronounced growth advantage (Cormier et al., 1998; Maeurer et al., 1996; Riker et al., 1999). On the contrary, if a TAA is required for tumor cell survival, such an antigen should not be prone to immune editing, because downregulation or loss of expression can be expected to reduce tumor cell growth.

Since, as outlined above, survivin seems to be important for tumor cell survival, downregulation or loss of survivin expression in tumor cells, as a possible mechanism of immune escape following vaccination, would hamper tumor progression and hence be beneficial to patients. Moreover, immunotherapeutic targeting of survivin should also target endothelial cells, providing an additional mechanism by which tumor growth could be controlled. Endothelial cells are genetically more stable than tumor cells
decreasing the chances for establishing escape mutants that avoid T cell recognition. Angiogenesis is a rate-limiting step in tumor development, since tumor size is generally limited to 1–2 mm³ in the absence of a sufficiently enhanced blood supply. Hence, survivin-based therapies target simultaneously tumor cells and the tumor vasculature. All together, these features make survivin a highly attractive target for T cell-based immune strategies against cancer (Fig. 2).

1. DENDRITIC CELL-BASED VACCINES

Dendritic cell (DC)-based vaccines have become one of the most powerful approaches to actively induce immunity and have been extensively used for cancer immunotherapy reaching phase III levels for several clinical trials. After the identification of survivin as a prominent TAA, several groups started to evaluate the ability of DCs to induce CTL immune responses against survivin. As stated above, several studies have shown that survivin-specific CTLs can be generated from peripheral blood of cancer patients and healthy individuals after in vitro stimulation with pulsed or transfected DCs (Andersen et al., 2001b; Schmidt et al., 2003; Schmitz et al., 2000; Zeis et al., 2003). The potential of using DC-based vaccines to elicit survivin-specific CTL-mediated protective immunity has been further studied in vivo using animal models. Using a model of A20 lymphoma, three weekly immunizations with survivin RNA transfected syngeneic DCs were shown to be sufficient to protect 83% of the mice, which then remained tumor free during several months (Zeis et al., 2003). Protein- or peptide-pulsed DCs have also been used in mouse tumor models. Siegel et al. (2003) showed that immunization with DCs that had been pulsed with Kd-restricted peptides could generate CTL responses and partial rejection of a lethal A20 lymphoma challenge. In another study, DCs transfected with human survivin encoding plasmids were employed and shown to yield efficient long-term protection when mice were subcutaneously challenged with GL261 glioma cells, but not when a mouse survivin plasmid was used or when mice were challenged intracerebrally with GL261 glioma cells rather than subcutaneously (Ciesielski et al., 2006). Others have tested in different experimental tumor models DCs transduced with adenoviruses encoding full-length human survivin (Ad-surv DC) with the (T34A) mutation to eliminate survivin antiapoptotic activity (Nagaraj et al., 2007). Immunization using Ad-surv(T34A) DCs generated specific CTL responses against several predicted epitopes. Total (adenocarcinoma MC38) or partial (thymoma EL4 and sarcoma MethA) tumor protection in prophylactic settings were observed, although the survival follow-up was only evaluated for up to 40 days. No significant protection was observed with Ad-surv(T34A) DCs in therapeutic settings. Evidence indicating that CD4⁺ T cell responses are
important for survivin-specific CTL-mediated tumor protection was obtained using DCs pulsed with a 15-mer class II peptide (surv53–67) that contains an internal class I epitope (surv57–64) (Ciesielski et al., 2008). Immunization using DCs pulsed with the 15-mer peptide generated CD8+ CTLs, as well as CD4+ T cell helper responses and generated significantly more effective rejection of preestablished GL261 tumor implants compared to DCs pulsed with the core class I peptide (surv57–64). The clinical relevance of this study resides in the fact that the sequence of the larger peptide is completely conserved between human and mouse and represents a human survivin CD4 epitope (Vence et al., 2007), whereas the class I core peptide is a HLA-A24-restricted CTL epitope (Bachinsky et al., 2005).

In vivo targeting of DCs represents an attractive strategy to improve vaccine potency. Immunization based on DEC205 receptor-mediated targeting of survivin to DCs showed that efficient CD4+ T cell responses can be generated using xenogeneic human survivin, but not the mouse survivin protein (Charalambous et al., 2006). These results indicate that the use of a xenogeneic survivin sequence may be important for enhancing CD4+ T cell responses when employing immunization strategies that include the full-length survivin gene or protein. However, this immunization strategy did not generate CD8+ T cell responses or tumor protection against the A20 lymphoma model. The lack of tumor protection observed in the absence of survivin-specific CTLs suggests that CD8+ T cell responses are crucial in mediating tumor rejection elicited by survivin-based vaccines. Another strategy for in vivo targeting of DCs present in secondary lymphatic organs, such as Peyer’s patches, is the oral administration of attenuated bacteria as a genetic immunization approach (Xiang et al., 2005). These authors used an attenuated strain of Salmonella typhimurium carrying a bicistronic plasmid that allowed for simultaneous expression of full-length murine survivin antigen fused to a mutant ubiquitin (Ub-surv) and the secretory chemokine CCL21, both under the control of different promoters. After three oral immunizations with this vaccine, efficient protection against D121 murine Lewis lung carcinoma was observed in prophylactic settings, as well as in therapeutic experimental metastasis assays. The efficacy of this vaccine was dramatically impaired when either the Ub-surv fusion or CCL21 vaccines were used alone. In both instances, despite detectable antitumor effects, disseminated pulmonary metastases were observed. As for the antitumor effects, the Ub-surv/CCL21 vaccine was considerably more efficient in inducing in vitro cytotoxicity, as well as tumor cell apoptosis. Similarly, flow cytometry analysis showed that the Ub-surv/CCL21 vaccine increased the proportion of CD3+ cells expressing activation markers, CD4+ T cells expressing IL-2, CD8+ T cells expressing IFN-γ, and upregulated costimulatory molecules in DCs. The in vitro characterization of the immune responses was
performed in immunized animals that had received tumor cell inoculation, which may make it difficult to determine the net effect of the immunization *per se*. Later, using the same approach, this group coexpressed survivin and the NKG2D ligand H60 and observed improved NK and CD8+ T cell-mediated protection against tumor formation using either breast or colon carcinoma models (Zhou *et al.*, 2005). This study highlights the potential benefit of activating both innate and adaptive arms of the immune system in order to efficiently induce survivin-mediated antitumor protection.

2. NAKED DNA VACCINES

DNA vaccination is a simple, safe, and attractive approach to deliver gene-encoded antigens that upon expression *in vivo* are presented by APCs to generate T- and B-cell responses (Feltquate, 1998; Gurunathan *et al.*, 2000). DNA vaccines have several advantages over other immunization strategies. First, the desired antigen-encoding plasmid is easy to generate and modify subsequently. Second, DNA vaccines are fairly inexpensive to produce and purify in large quantities and the same production platform can be used for plasmids encoding any protein antigen, obviating expensive purification procedures for each particular antigen. Third, such vaccines are very stable and can be easily stored long term (cold-chain not required). Fourth, the plasmids employed are noninfectious and there is virtually no genomic integration, hence few side effects are expected. Fifth, the antigen is processed and presented by host cells with no restriction or requirement of certain haplotypes as compared with peptide-based vaccines. Finally, the plasmids employed contain danger signals that activate different innate immune receptors and boost specific adaptive immune responses. Different groups have made efforts to target survivin using DNA vaccines. In our studies, we provided proof of principle by showing that intramuscular immunization of naked DNA encoding for human survivin could generate CD8+ T cell responses, as determined by *ex vivo* stimulation of spleen cells with syngeneic P815 tumor cells and intracellular IFN-γ staining (Lladser *et al.*, 2006). Similar cellular responses were obtained using a plasmid encoding a secreted form of survivin TAA, indicating that cross-presentation is an important mechanism involved in inducing T cell responses after intramuscular inoculation of plasmids. In addition, survivin-specific humoral responses were observed when survivin plasmid was coadministrated with a plasmid encoding for the murine granulocyte-macrophage colony-stimulating factor (GM-CSF). Immunoglobulin isotyping showed that IgG2a responses were favored, indicating a predominant Th1-polarized immune response. Another publication also showed that antibody responses were elicited using a different plasmid construct where a secreted form of mouse survivin carrying the
T34A mutation fused to the DC-binding domain of heat shock protein 70 was used (Decker et al., 2006). In this study, antitumor efficacy against colon adenocarcinoma CT26 cells revealed a reduction in tumor volume, although no long-term mouse survival data was provided. Later, using naked DNA vaccines, Zhu et al. observed slightly enhanced survival in mice challenged with either A20 lymphoma or Panc02 pancreatic adenocarcinoma cells following immunization with plasmids encoding either human or mouse survivin (Zhu et al., 2007). The observed antitumor effects correlated with CD3+ lymphocyte infiltration at the tumor site; however, the authors were unable to detect specific CD8+ T cell responses by Elispot using previously described epitopes. Although these efforts targeting tumors using naked survivin DNA vaccines delivered by classical intramuscular injection have shown antitumor effects in vivo, only modest tumor protection was reported, which motivated us to use a more efficient vaccination approach. Recently, we used intradermal electroporation (EP) to deliver our survivin DNA vaccine. This method allows efficient DNA uptake, high levels of antigen expression and enhanced cellular immune responses, as well as the induction of several cytokines and chemokines, thereby increasing the potency of DNA vaccines (Roos et al., 2006, 2009). Survivin DNA EP elicited CD8+ T cells specific for the survivin peptide (surv20–28) that we defined as a H-2 Db-restricted epitope (Lladser et al., 2010) and previously identified as an epitope restricted to HLA-A24 (Bachinsky et al., 2005). DNA vaccine-induced surv20–28-specific CD8+ T cells displayed cytolytic activity in vitro and in vivo. Furthermore, survivin DNA EP suppressed angiogenesis in an in vivo matrigel assay and conferred tumor protection against highly aggressive B16 melanoma cells (Lladser et al., 2010). Moreover, the immunogenicity of this survivin DNA vaccine could be further potentiated by codelivering the gene encoding an innate immune receptor that recognizes cytosolic DNA, termed DNA-dependent activator of interferon regulatory factors, DAI (also known as ZBP1 and DLM-1). The use of DAI as a genetic adjuvant enhanced the frequency of survivin-specific IFN-γ-producing CD8+ T cells more than fivefold compared to the survivin DNA vaccine alone. These results were almost identical for two different epitopes surv20–28 and surv57–64. Interestingly, this increase was also observed for CD4+ T helper 1 responses to the MHC class II-restricted survivin epitope surv53–67 and correlated with higher tumor protection in vivo (Lladser et al., 2011). In summary, survivin DNA vaccination is an attractive approach with great potential for developing a universal cancer vaccine that, however, still requires further optimization and testing especially in therapeutic settings. The usage of potent delivery systems, such as in vivo EP, also merits further attention in this context.
C. Survivin-Based Vaccines in the Clinic

As stated in the previous sections, survivin-specific immune responses have been shown to spontaneously arise in cancer patients and survivin-specific CTLs are generated in vitro after stimulation of human lymphocytes with survivin-loaded DCs. Moreover, studies in animal models have demonstrated the potential to induce in vivo survivin-specific protective CTL immunity. Taken together both clinical and preclinical studies have motivated clinicians to test survivin vaccines in humans. The first clinical trial was performed on five phase IV melanoma patients that received autologous DCs pulsed with a survivin-derived HLA-A2-restricted synthetic peptide (surv96–104) modified with a threonine to methionine substitution at position 97 (LMLGEFLKL), which represents a better anchor residue with enhanced binding to HLA-A2 molecules (Otto et al., 2005). The repetitive administration of survivin peptide-loaded DCs was shown to be safe and neither major toxic effects nor signs of autoimmunity were detected. Four of the five treated patients mounted T cell responses to the surv96–104 epitope as measured by the ELISPOT assay. Although not detectable in direct ex vivo analyses, T cell responses could be readily measured after a 10-day in vitro peptide-stimulation that permits amplification of rare T cell populations. Remarkably, surv96–104-specific CTLs infiltrated both soft tissue and visceral metastases, as detected by in situ surv96–104/HLA-A*0201 multimer staining, a feature that is often associated with favorable clinical outcome, such as enhanced survival (Nakano et al., 2001; Sato et al., 2005). Although no definitive conclusions regarding the clinical efficacy of survivin-loaded DC vaccination could be drawn from this study, enhanced long-term survival was observed in the four patients that mounted survivin-specific T cell responses. The main conclusion of this study is that survivin-specific T cells with the ability to infiltrate metastatic lesions can be induced in patients with advanced melanoma in the absence of toxic and autoimmune side effects associated with the use of this survivin-based DC vaccine (Otto et al., 2005). The first case of successful application of a survivin-based vaccine was observed in a patient with liver metastasis of pancreatic cancer that was immunized with monthly subcutaneous injections of the modified peptide surv96–104 in Montanide. The 72-year old male patient showed a reduction in tumor markers to normal levels, followed by partial remission that then proceeded to complete remission 14 months after vaccination had started, whereby no evidence of disease was detectable during the last 8 months of treatment (Wobser et al., 2006). Notably, this objective clinical response correlated with the induction of surv96–104-specific CTLs, as measured by ELISPOT assay, and multimer staining.
after *in vitro* peptide-stimulation. Unfortunately, the patient suffered a fatal relapse after the vaccinations ceased.

In another phase I trial, patients with advanced colorectal cancer were vaccinated with a HLA-A24-restricted peptide derived from the exon 2B-containing splice variant of survivin (surv2B<sub>80–88</sub>, AYACNTSTL). After 6 immunizations given at 2-week intervals, no severe adverse effects were reported, but grade 1 and 2 toxicity levels were detected in 3 out of the 15 patients treated (Tsuruma *et al.*, 2004). Tumor marker levels decreased transiently during the vaccination period in 6 patients. However, only marginal clinical and T cell responses were observed. A similar protocol was applied to patients with advanced urothelial (Honma *et al.*, 2009) and breast cancer (Tsuruma *et al.*, 2008). A group of patients from the later study received four injections of the peptide in combination with incomplete Freund’s adjuvant (IFA). The adjuvant enhanced the proportion of patients that developed survivin-specific T cell responses although no clinical responses were observed. In summary, survivin-based vaccination has been demonstrated to be safe and to possess therapeutic potential in cancer patients. However, further clinical trials are required to evaluate whether the combination with adjuvants or drugs improves the immunological and therapeutic efficacy. Indeed, some studies have used survivin as part of a cocktail of tumor-associated antigens to maximize the potential efficacy of the vaccines (Berntsen *et al.*, 2008; Hirschowitz *et al.*, 2007; Trepiakas *et al.*, 2010).

### IV. POTENTIAL PITFALLS ASSOCIATED WITH SURVIVIN-BASED THERAPIES

#### A. Survivin Expression in Nontransformed Adult Tissues

Survivin is regarded as one of the most tumor-specific proteins, due to its high level of expression in human cancers and general absence in most normal tissues. However, there is mounting evidence indicating that survivin is also expressed in nontransformed adult cells characterized by self-renewal and active proliferation (reviewed in Fukuda and Pelus (2006)). Survivin expression in normal adult tissues was originally reported to be restricted to thymus and placenta (Ambrosini *et al.*, 1997). Since then a number of studies have demonstrated that survivin is expressed in other human tissues, including testis (Kobayashi *et al.*, 1999), keratinocytes (Chiodino *et al.*, 1999), endometrium (Konno *et al.*, 2000), vascular endothelial cells (O’Connor *et al.*, 2000b; Tran *et al.*, 1999), colon crypt epithelial cells (Zhang *et al.*, 2001), T cells (Kornacker *et al.*, 2001), CD34<sup>+</sup> hematopoietic stem cells (Fukuda and Pelus, 2001), cervical mucosa
(Frost et al., 2002), gastric mucosa (Chiou et al., 2003; Valenzuela et al., 2010), neutrophils (Altznauer et al., 2003), and ovaries (Kumazawa et al., 2005). In most cases, survivin is not present under resting conditions and is upregulated upon exposure to stress, or in response to cytokines or proliferative stimuli, suggesting that survivin plays physiological roles in these tissues. In contrast to cancer cells, survivin expression is cell cycle-regulated in normal adult cells and is implicated there predominantly in controlling proliferation rather than apoptosis. In this context, it should be noted that the mitochondrial pool of survivin, present in tumor cells, which inhibits apoptosis and promotes tumorigenesis (Dohi et al., 2004), has not been described in normal adult cells. Highly relevant is that survivin expression is significantly lower in nontransformed adult tissues compared to tumor cells.

Although survivin is expressed transiently and at lower levels in normal adult cells, exhaustive studies are needed to control whether survivin-based cancer therapies may also affect healthy tissues. Some efforts have been made in this direction. For instance, infection of proliferating human fibroblasts, endothelial or smooth muscle cells with an adenoviral vector carrying the T34A survivin mutant had no impact on cell viability or proliferation and systemic administration of this recombinant adenovirus did not elicit any toxic side effects (Mesri et al., 2001b). However, in another study, intratumoral injection of T34A survivin adenovirus did induce apoptosis in tumor-associated endothelial cells (Blanc-Brude et al., 2003), raising the concern that survivin targeting may also affect normal endothelial cells. Another agent used to target survivin function is the peptide shepherdin, a survivin-derived peptide that disrupts survivin/Hsp90 interaction and destabilizes several Hsp90 client proteins. This peptide did not affect adversely different hematopoietic progenitors at concentrations where tumor cell viability was profoundly compromised, although increasing concentrations could generate some adverse effects (Plescia et al., 2005). Such effects can be expected since stable ablation of survivin severely compromises hematopoietic progenitors (Fukuda et al., 2002, 2004; Gurbuxani et al., 2005). Therefore, long-term, systemic exposure to agents that target survivin may induce severe adverse effects in the hematopoietic system and careful in vivo studies are needed to control for potential complications associated with any survivin-based therapy.

B. Oncogenic Potential Associated with Survivin Gene Transfer

Another issue to consider, when using survivin gene-based vaccines in the clinic, is the oncogenic potential associated with introducing a construct encoding the full-length survivin gene into normal cells. Survivin is
expected to exert its biological function once expressed \textit{in vivo} and, although survivin is not considered an oncogene \textit{per se} because overexpression does not transform normal cells, excessive presence may contribute to the phenotype of cancer cells. Studies using survivin-transgenic mouse models have provided valuable information in this regard. Transgenic thymocytes from mice where survivin is under the control of lymphocyte-specific protein tyrosine kinase (lck) promoter, displayed hyperproliferation in response to phorbol-12-myristate-13-acetate (PMA) and ionomycin but not to anti-CD3 antibody stimulation (Hikita \textit{et al.}, 2002). Moreover, T cell development and apoptosis of thymocytes were not affected in this model. In a recent study, transgenic mice that express survivin in hematopoietic cells under the control of the GATA-1 promoter developed hematologic malignancies at an increased rate and with shorter latency after treatment with the DNA alkylating agent \textit{N}-ethyl-nitrosourea (ENU) as compared with wild-type mice (Small \textit{et al.}, 2010). However, survivin overexpression alone was not sufficient to induce malignant transformation in different hematopoietic lineages (McCran \textit{et al.}, 2008; Small \textit{et al.}, 2010). Another model that is relevant to the study of side effects associated with introducing DNA-encoded survivin for immunization purposes is a transgenic mouse where survivin expression is under the control of the keratin-14 promoter, which is active in the skin. In this model, survivin expression counteracted UVB-induced apoptosis, as expected, but the skin developed normally without histological abnormality or hyperplasia (Grossman \textit{et al.}, 2001a). Moreover, proliferation was not altered under normal conditions or after UVB exposure or treatment with phorbol esters. Further studies using this same model have shown no increased or even reduced premalignant lesion and tumor onset after chemical induction (Allen \textit{et al.}, 2003; Thomas \textit{et al.}, 2007). None-the-less, survivin expression could promote later events in the carcinogenic process in conjunction with other cancer-related mutations. However, analysis of mouse skin electroporated with DNA vaccines has shown that expression of an immunogenic, tumor-associated antigen able to induce CTL responses is no longer detected after 21 days (Roos \textit{et al.}, 2009). These observations tend to make it unlikely that survivin will contribute to late events of carcinogenesis. Therefore, we consider the risk of malignant transformation associated with survivin transfection of normal skin cells to be very low. Despite such encouraging evidence, for future clinical trials it may be worth considering utilizing gene constructs encoding nonfunctional survivin variants.

C. Risks of Autoimmunity

An important consideration for survivin-based immunotherapies is the development of autoimmunity. Survivin-specific T cells have the
potential to recognize nontransformed, survivin-expressing cells and, consequently, trigger undesired long-term effects. Some studies have analyzed this potential pitfall. In a preclinical study, the potential adverse effects on endothelial cells associated with the induction of survivin-specific CTLs were evaluated. Effective antitumor CTL immunity by means of a survivin encoding DNA vaccine was shown to be safe for healthy tissues (Xiang et al., 2005). This vaccine suppressed tumor-associated angiogenesis without affecting wound healing or fertility. Human survivin-specific CTL immune responses induced in vitro by stimulating with DCs infected with a survivin-encoded adenovirus lysed survivin peptide-pulsed target cells, as well as breast cancer cells expressing endogenously survivin, in an in vitro cytotoxicity assay. In contrast, induced survivin-specific CTLs did not lyse survivin-expressing CD34+ hematopoietic progenitors, unless these were exogenously loaded with survivin peptides (Pisarev et al., 2003). Similarly, survivin-specific CTLs induced with peptide-pulsed DCs did not lyse activated DCs, B- and T-lymphocytes endogenously expressing survivin that were used as targets. Activated B- and T-cells became susceptible to CTL-mediated lysis only after pulsing them with survivin peptides (Schmidt et al., 2003). However, a recent study showed that human T cells genetically engineered to express a high affinity survivin-specific TCR could lyse survivin-expressing tumor cell lines and also cause extensive apoptosis of HLA-matched T cells expressing high levels of survivin upon in vitro activation (Leisegang et al., 2010). Expression of survivin and subsequent presentation of survivin-derived epitopes on the cell surface of activated lymphocytes led to their recognition and fratricide killing by survivin-specific TCR-engineered T cells in a MHC-restricted fashion and irrespective of the specificity of the target lymphocytes. These observations indicate that MHC-restricted fratricide killing may limit the efficacy of immunotherapies based on survivin CTLs by eliminating survivin-expressing lymphocytes at sites where these cells accumulate either during induction (lymph nodes) or activation (tumor). This anticipated drawback contrasts with reports indicating that survivin-specific CTLs can be found systemically or infiltrating tumors of patients with several kinds of cancer (Andersen et al., 2001a,b; Casati et al., 2003; Coughlin et al., 2006; Grube et al., 2007; Reker et al., 2004a,b; Siegel et al., 2004). Such cells even persisted several years in a melanoma patient that had undergone complete remission after receiving IL-2-based immunotherapy (Hadrup et al., 2006). These studies, in addition to the lack of toxicity observed in the first clinical trials using survivin-based vaccines, suggest that the low endogenous levels of survivin expressed in nontransformed cells are not sufficient to make them suitable targets for survivin-specific CTLs.
V. CONCLUDING REMARKS

Survivin is a member of the IAP-family that plays a crucial role in preventing cell death and promoting proliferation. This protein is frequently upregulated in tumors, despite the existence of elaborate mechanisms that control its presence at multiple levels. Loss of function in tumor suppressors and gain of function in oncogenes contribute to the constitutive expression of survivin observed in most cancer cells. Since augmented survivin levels greatly enhance cancer cell survival and malignancy, many strategies have been developed that seek to target survivin in cancer treatment (Fig. 2). Such approaches include the use of survivin antisense molecules, small-molecule inhibitors (YM155), dominant negative mutants (C84A, T34A), and peptide antagonists (shepherdin). Many of these treatments are now being evaluated in clinical trials either alone or in combination with chemotherapeutic drugs.

Promising results have been obtained using experimental DC- and DNA-based cancer vaccines targeting survivin indicating that survivin-based immunotherapy has considerable potential against tumors. However, successful therapeutic intervention using these approaches and the development of relevant therapeutic tumor models still remain a major challenge. DNA vaccines targeting survivin have been shown to induce antitumor activity in vivo. However, vaccines encoding the survivin antigen alone seem to be relatively inefficient in inducing protective antitumor responses. Hence, optimization of vaccination with plasmids encoding the survivin gene remains an area of great potential that needs to be explored further. Modifications of the encrypted antigen that improve immunogenicity, DC uptake, and/or antigen presentation are some of the advantages of DNA vectors that need to be pursued. In addition, improvements of DNA delivery methods, such as in vivo EP have progressed tremendously in recent years and led to promising results that are being tested currently in several clinical trials.

The variety of survivin-targeting approaches described here holds great promise for the development of successful cancer therapies. However, many more trials are required to reveal whether or not survivin is truly the Achilles’ heel of cancer.

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