

The Universal Character of the Tumor-Associated Antigen Survivin

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Abstract Survivin is expressed in most human neoplasms, but is absent in normal, differentiated tissues. Survivin is a bifunctional inhibitor of apoptosis protein that has been implicated in protection from apoptosis and regulation of mitosis. Several clinical trials targeting survivin with a collection of different approaches from small molecule antagonists to immunotherapy are currently under way. With regard to the latter, spontaneous anti-survivin T-cell reactivity has been described in cancer patients suffering from a huge range of cancers of different origin, e.g., breast and colon cancer, lymphoma, leukemia, and melanoma. Thus, survivin may serve as a universal target antigen for anticancer immunotherapy. Accordingly, down-regulation of survivin as a means of immune escape would severely inflict the survival capacity of tumor cells, which highlights this protein as a prime target candidate for therapeutic vaccinations against cancer. Data from several ongoing phase I/II trials targeting survivin for patients with advanced cancer will provide further information about this idea.

Background

The function of survivin. Most malignancies are characterized by defects in apoptosis signaling. Among the regulators of apoptosis implicated in cancer cell survival, Bcl-2 proteins are thought to regulate the mitochondria permeability transition by inhibiting or promoting cytochrome *c* release, whereas members of the inhibitors of apoptosis (IAP) gene family function as endogenous inhibitors of caspases (1). Eight human IAP family members have been identified thus far. They are c-IAP1, c-IAP2, XIAP, NAIP, survivin, apollon, ML-IAP/livin, and ILP-2 (2). Survivin has attracted attention as a unique member of the IAP gene family with a potential dual role in apoptosis inhibition and regulation of mitosis (3).

The role of survivin in the regulation of mitosis has recently become better understood, and linked to multiple spindle microtubule functions and mitotic checkpoints (4, 5). Thus, in normal cells, survivin is preferentially expressed at mitosis in a cell cycle-dependent manner and is physically associated with the mitotic apparatus. Survivin is essential for the proper completion of various stages of cell division, from centrosomal functions to proper kinetochore attachment to spindle formation, potentially via the regulation of microtubule dynamics/stability.

In contrast, despite extensive experimental evidence *in vitro*, and in transgenic animals *in vivo* (3), the precise mechanism(s) by which survivin interferes with apoptosis has not been elucidated. Survivin counteracts cell death by interfering with

caspase-9 processing (6), the upstream initiation of the intrinsic (mitochondrial) pathway of apoptosis. Furthermore, cytoprotection by survivin is more selective than that by other IAPs, and is specifically targeted at the initiation of mitochondrial apoptosis to prevent caspase-9 activation (1).

Expression of survivin. Survivin is abundantly and ubiquitously present in development, is undetectable in most adult tissues, and is prominently re-expressed in virtually every human cancer. Thus, one of the most significant features of survivin is its preferential expression in tumor versus normal tissues. Even though a few normal cells do express survivin, e.g., thymocytes, CD34+ bone marrow-derived stem cells, and basal colonic epithelial cells (3), under physiologic conditions, survivin is undetectable in most terminally differentiated normal tissues (7). In normal cells, survivin expression at G₂-M phase is followed by a rapid decline at G₁ phase. However, it has been suggested that the *survivin* gene might be globally deregulated in transformed cells, leading to overexpression at all phases of the cell cycle, not just during the G₂-M phase (3). A global deregulation of the *survivin* gene mediated by oncogenes, including signal transducers and activators of transcription 3, E2F, or mutated Ras, or by loss of tumor suppressors, like p53 or the adenomatous polyposis coli protein, accounts for the selective expression of survivin in cancer (1).

Survivin is overexpressed in almost all cancers including lung, colon, breast, pancreas, stomach, liver, ovary and prostate cancer, as well as in melanoma and hematopoietic malignancies (8–10). Recently, it was reported that expression of survivin and the inhibitory T cell ligand B7-H1 could be used to predict clear cell renal cell carcinoma tumor aggressiveness (11). Data from a large analysis of human transcripts revealed survivin as the fourth most highly expressed protein in human cancer tissue compared with normal tissue (12). The extremely high expression of survivin in cancer carries prognostic and predictive implications and, through molecular profiling, is consistently associated with advanced disease, high grade, abbreviated survival, resistance to therapy, and accelerated

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recurrences. Clinical exploitation of *survivin* for cancer molecular diagnostics is under way, with its inclusion as 1 of 16 genes predictive of recurrences in breast cancer (13) and as a urine biomarker in bladder cancer (14).

Clinical Translational Advances

Survivin in therapy. Essentially all cytotoxic anticancer drugs, e.g., microtubule-binding drugs, DNA-damaging agents, and nucleosides currently in clinical use, induce the apoptosis of malignant cells. Up-regulation of IAP proteins plays a vital role in resistance to chemotherapy and radiotherapy (15). Thus, overexpression of survivin in cancer cells is associated with decreased overall survival (16–18), an increased rate of recurrences, and a reduced apoptotic index of neoplastic cells *in vivo* (3). Although conventional drugs are important weapons in the treatment of cancer, new classes of targeted therapeutics are emerging based on strategies that rely on a deeper understanding of the molecular mechanisms that underlie the phenomenon of apoptosis. In view of this, strategies aimed at inhibiting the expression or function of antiapoptotic proteins has gained considerable attention (19, 20). Thus, although survivin overexpression leads to increased resistance to apoptotic stimuli such as chemotherapeutic agents or ionizing radiation therapy, antisense-mediated silencing of survivin sensitizes the cells to these interventions (21). Supported by a favorable safety profile, the original survivin antisense oligonucleotide has now completed a phase I trial in patients with advanced cancers, and a phase II trial has been announced. A parallel strategy to suppress survivin levels in tumor cells involved RNA interference (22) or hammerhead ribozymes (23). These reagents produced a phenotype similar to antisense and passed proof-of-concept with inhibition of tumor growth in xenograft models. Another strategy is the use of synthetic peptides that compete with caspases for binding to IAPs, which have been shown to sensitize tumor cell lines to apoptosis induced by cytotoxic anticancer drugs (24). Several clinical trials using these reagents are under way. One of these molecules, tetra-*O*-methyl nordihydroguaiaretic acid was shown to function by suppressing Sp1-dependent *survivin* gene expression, resulting in the concomitant activation of mitochondrial apoptosis in tumor cells (25).

In addition to the abovementioned means of targeting survivin, immunomediated tumor destruction is emerging as an interesting modality to treat patients with cancer. A significant effort in this field is aimed at the elucidation of tumor-associated antigens. Accordingly, down-regulation of survivin would severely inflict the survival capacity of tumor cells, which highlights this protein as a prime target candidate for therapeutic vaccinations against cancer because it is not subject to immune selection, i.e., the selection of cancer cells not expressing the vaccination target. In this regard, several recent reports have—as we will see below—shown that survivin is among the tumor antigens that serve as targets for immunomediated tumor destruction in patients with cancer.

Presentation of survivin-derived peptides by cancer cells to the immune system. Cellular immunity is largely based on T lymphocytes. A T cell expresses a unique antigen-binding molecule called the T-cell receptor on the cell surface. In contrast to membrane-bound antibodies on B cells, which can recognize antigen alone, the vast majority of T-cell receptors

recognize a complex ligand, comprising an antigenic peptide bound to MHC-derived molecules (26). The formation of peptide-MHC complexes requires that a protein antigen is degraded by a sequence of events called antigen processing. CTLs recognize antigenic peptides in the context of MHC class I molecules. The generation of mature, peptide-loaded class I molecules at the cell surface requires the coordination of three essential processes: first, the degradation of proteins to peptides in the cytosol; second, the translocation of peptides across the endoplasmic reticulum membrane; and third, the assembly of MHC class I/peptide heterotrimers and their transport to the cell surface (Fig. 1; ref. 27).

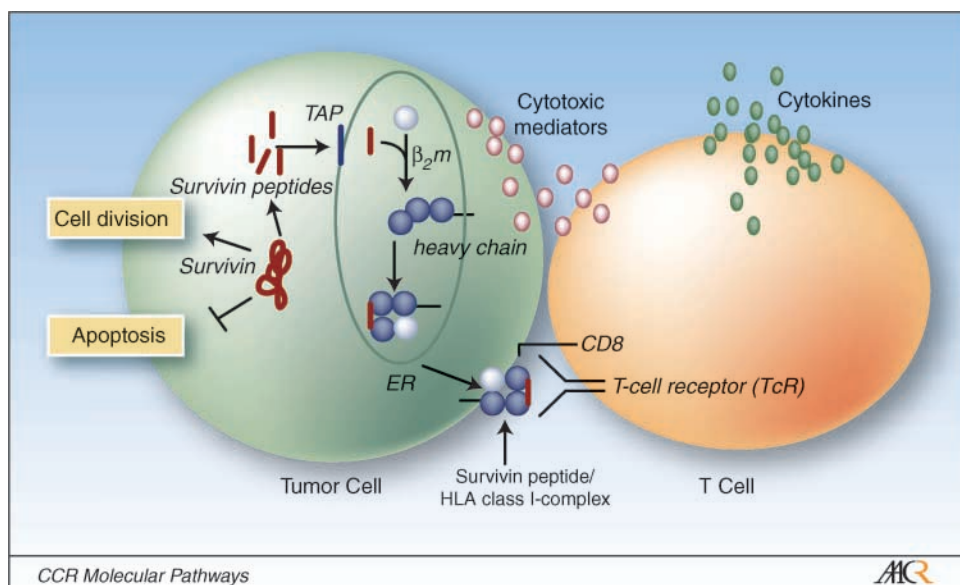
CTLs have a vital function in monitoring the cells of the body and eliminating those cells displaying the respective antigen. CTL are thought to provide the major part of the immune surveillance against tumors by virtue of their ability to detect quantitative and qualitative antigenic differences in transformed cells (28). Oncogenic alterations result in an altered protein repertoire inside the cell, e.g., high expression of survivin. Subsequently, class I MHC molecules sample survivin peptides derived from protein degradation inside the cell and present these at the cell surface (Fig. 1). When a T cell encounters antigen in the context of an HLA molecule, it undergoes clonal expansion and differentiates into various effector as well as memory T cells.

Survivin-specific immune responses. In the February 2007 issue of *Clinical Cancer Research*, Grube and colleagues very convincingly describe that CD8+ T cells react against the survivin antigen in patients with multiple myeloma (29). Likewise, Coughlin et al. recently reported that children with high-risk neuroblastoma harbor robust cellular immune responses to survivin at the time of diagnosis (30). Both studies underline the generality of the tumor antigen survivin. In this regard, spontaneous anti-survivin T-cell reactivity has previously been described in cancer patients suffering from breast cancer, colon cancer, lymphoma, leukemia, and melanoma (31–36).

To explore the lytic capacity of survivin-specific T cells *ex vivo*, we isolated such cells by means of magnetic beads coated with MHC/peptide complexes. As predicted, these survivin-reactive T cells were capable of lysing HLA-matched tumor cells of different tissue origin, including breast cancer cells and melanoma (32). These observations were confirmed by Schmidt et al. who showed that survivin-specific CTL generated from healthy donors or patients with leukemia elicited cytolytic activity against tumor cells endogenously expressing the survivin protein, including renal cell carcinomas, breast cancer, colon cancer, melanoma, and multiple myeloma cell lines, as well as primary malignant cells from patients with different leukemias (37). Coughlin et al. used CD40-activated B cells transfected with whole tumor RNA, which induced survivin-specific T cells *in vitro* that lysed HLA-matched neuroblastoma cells but not autologous benign cells (38). Hence, survivin is among the targeted antigens in vaccination strategies using whole tumor cells as an antigen source.

There is consensus that the induction of efficient and powerful T cell responses requires proper activation of the T cell. However, it is equally important that the T cells acquire the ability to home to the site of action. *In situ* immunohistochemistry using multimerized HLA/survivin-peptide complexes disclosed that survivin-specific CTL could readily be

Fig. 1. Principle of the processing pathway of survivin peptides by tumor cells and the subsequent recognition by CD8⁺ CTLs. Survivin is highly expressed in cancer cells. It works as an IAP protein, and in addition, is important during cell division. Several clinical trials including small molecule antagonists and antisense oligonucleotides are targeting the function of survivin. Furthermore, as T cells in patients with cancer specifically recognize survivin, immunomediated tumor destruction is emerging as an interesting modality to treat patients with cancer. The epitopes recognized by the T cells are short survivin – derived peptides resulting from the degradation of intracellular survivin protein, which are presented on the cell surface of class I HLA molecules. T cells receive an activation signal through their T-cell receptor complex, leading to a variety of functional consequences, including the release of cytokines and cytotoxic molecules.



detected in the tumor microenvironment, both in primary tumors and metastases of patients with melanoma as well as in breast cancer lesions (32, 33). The combined detection of survivin-specific T cells in blood and tumor lesions indicates that these cells are capable of circulating and homing to the effector site. This is a significant finding because several clinical reports have indicated the existence of a functional dissociation between local and systemic anti-melanoma T cell responses (39, 40). Interestingly, Coughlin and coworkers describe a high frequency of circulating, functional survivin-specific CTLs, whereas intratumoral T cells were strikingly rare. Thus, survivin-based immunotherapy should indeed focus on T cell trafficking into tumor nests (30).

Immunologic clinical advances. Taken together, these pre-clinical studies have justified clinical testing of survivin for anticancer vaccination. Consequently, Survivin-directed immunotherapy has quickly been moved to the clinic. Recently, the first therapy-induced T cell responses against survivin were described. In a compassionate use setting, heavily pretreated patients with stage IV melanoma were vaccinated with an HLA-A2-restricted survivin epitope presented by autologous dendritic cells (41). These patients mounted strong T cell responses to this epitope as measured by ELISPOT assay. Furthermore, *in situ* peptide/HLA-A2 multimer staining revealed the infiltration of survivin-reactive CD8⁺ T cells into the soft tissue metastases of vaccinated patients. Notably, no vaccination-associated toxicity was observed. Hence, it is feasible to induce T cell responses against survivin; even in

late stage tumor patients, and these vaccinations are well tolerated. In that regard, Pisarev et al. recently showed that survivin-directed CTL do not affect hematopoietic colony formation of CD34⁺-purified progenitor cells (42). In addition, in a patient with melanoma in complete remission following interleukin 2-based immunotherapy, a longitudinal examination of anti-survivin reactivity was made exceeding 7 years. Survivin-specific T-cell reactivity was found at all time points examined over the 7-year period. The data showed that anti-survivin T cells may persist in the periphery for extended periods in the absence of clinical manifestation of disease as well as autoimmunity (43). Finally, when used in an oral DNA vaccine, the survivin-directed immune response affected both tumor cells and tumor-associated angiogenesis, eradicating pulmonary metastases without toxicity, including wound healing or fertility in preclinical studies (44).

The first example of a successful application of survivin-based vaccination in the clinical setting was recently described in a patient with pancreatic cancer (45). This patient had a complete remission of liver metastasis under vaccination with a HLA-A2-restricted survivin peptide together with an adjuvant. Data from several ongoing phase I/II trials targeting survivin for patients with advanced cancer will provide further information towards this notion. In this regard, several survivin-based vaccination trials are currently ongoing at different institutes, e.g., Istituto Nazionale Tumori (Milan, Italy), Würzburg University Hospital (Würzburg, Germany), and Herlev University Hospital (Herlev, Denmark).

References

- Salvesen GS, Duckett CS. IAP proteins: blocking the road to death's door. *Nat Rev Mol Cell Biol* 2002;3: 401–10.
- Reed JC. Apoptosis-based therapies. *Nat Rev Drug Discov* 2002;1:111–21.
- Altieri DC. Validating survivin as a cancer therapeutic target. *Nat Rev Cancer* 2003;3:46–54.
- Yang D, Welm A, Bishop JM. Cell division and cell survival in the absence of survivin. *Proc Natl Acad Sci U S A* 2004;101:15100–5.
- Li F, Ambrosini G, Chu EY, et al. Control of apoptosis and mitotic spindle checkpoint by survivin. *Nature* 1998;396:580–4.
- LaCasse EC, Baird S, Korneluk RG, MacKenzie AE. The inhibitors of apoptosis (IAPs) and their emerging role in cancer. *Oncogene* 1998;17:3247–59.
- Altieri DC, Marchisio PC, Marchisio C. Survivin apoptosis: an interloper between cell death and cell proliferation in cancer. *Lab Invest* 1999;79:1327–33.
- Ambrosini G, Adida C, Sirugo G, Altieri DC. Induction of apoptosis and inhibition of cell proliferation by survivin gene targeting. *J Biol Chem* 1998;273: 11177–82.
- Adida C, Haioun C, Gaulard P, et al. Prognostic significance of survivin expression in diffuse large B-cell lymphomas. *Blood* 2000;96:1921–5.
- 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.

11. Krambeck AE, Dong H, Thompson RH, et al. Survivin and b7-1 are collaborative predictors of survival and represent potential therapeutic targets for patients with renal cell carcinoma. *Clin Cancer Res* 2007;13:1749–56.
12. Velculescu VE, Madden SL, Zhang L, et al. Analysis of human transcriptomes. *Nat Genet* 1999;23:387–8.
13. Giodini A, Kallio MJ, Wall NR, et al. Regulation of microtubule stability and mitotic progression by survivin. *Cancer Res* 2002;62:2462–7.
14. Smith SD, Wheeler MA, Plescia J, Colberg JW, Weiss RM, Altieri DC. Urine detection of survivin and diagnosis of bladder cancer. *JAMA* 2001;285:324–8.
15. Longley DB, Johnston PG. Molecular mechanisms of drug resistance. *J Pathol* 2005;205:275–92.
16. Islam A, Kageyama H, Takada N, et al. High expression of survivin, mapped to 17q25, is significantly associated with poor prognostic factors and promotes cell survival in human neuroblastoma. *Oncogene* 2000;19:617–23.
17. Swana HS, Grossman D, Anthony JN, Weiss RM, Altieri DC. Tumor content of the antiapoptosis molecule survivin and recurrence of bladder cancer. *N Engl J Med* 1999;341:452–3.
18. Kawasaki H, Altieri DC, Lu CD, Toyoda M, Tenjo T, Tanigawa N. Inhibition of apoptosis by survivin predicts shorter survival rates in colorectal cancer. *Cancer Res* 1998;58:5071–4.
19. Wacheck V, Selzer E, Gunsberg P, et al. Bcl-x(L) antisense oligonucleotides radiosensitize colon cancer cells. *Br J Cancer* 2003;89:1352–7.
20. Wang S, Yang D, Lippman ME. Targeting Bcl-2 and Bcl-XL with nonpeptidic small-molecule antagonists. *Semin Oncol* 2003;30:133–42.
21. Olie RA, Simoes-Wust AP, Baumann B, et al. A novel antisense oligonucleotide targeting survivin expression induces apoptosis and sensitizes lung cancer cells to chemotherapy. *Cancer Res* 2000;60:2805–9.
22. 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.
23. Pennati M, Binda M, Colella G, et al. Ribozyme-mediated inhibition of survivin expression increases spontaneous and drug-induced apoptosis and decreases the tumorigenic potential of human prostate cancer cells. *Oncogene* 2004;23:386–94.
24. Fulda S, Wick W, Weller M, Debatin KM. Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma *in vivo*. *Nat Med* 2002;8:808–15.
25. Chang CC, Heller JD, Kuo J, Huang RC. Tetra-O-methyl nordihydroguaiaretic acid induces growth arrest and cellular apoptosis by inhibiting Cdc2 and survivin expression. *Proc Natl Acad Sci U S A* 2004;101:13239–44.
26. Moss PA, Rosenberg WM, Bell JL. The human T cell receptor in health and disease. *Annu Rev Immunol* 1992;10:71–96.
27. Pamer E, Cresswell P. Mechanisms of MHC class I-restricted antigen processing. *Annu Rev Immunol* 1998;16:323–58.
28. Castelli C, Rivoltini L, Andreola G, Carrabba M, Renkvist N, Parmiani G. T-cell recognition of melanoma-associated antigens. *J Cell Physiol* 2000;182:323–31.
29. Grube M, Moritz S, Obermann EC, et al. CD8+ T cells reactive to survivin antigen in patients with multiple myeloma. *Clin Cancer Res* 2007;13:1053–60.
30. Coughlin CM, Fleming MD, Carroll RG, et al. Immunosurveillance and survivin-specific T-cell immunity in children with high-risk neuroblastoma. *J Clin Oncol* 2006;24:5725–34.
31. Andersen MH, Pedersen LO, Becker JC, thor Straten P. Identification of a cytotoxic T lymphocyte response to the apoptosis inhibitor protein survivin in cancer patients. *Cancer Res* 2001;61:869–72.
32. Andersen MH, Pedersen LO, Capeller B, Brocker EB, Becker JC, thor Straten P. Spontaneous cytotoxic T-cell responses against survivin-derived MHC class I-restricted T-cell epitopes *in situ* as well as *ex vivo* in cancer patients. *Cancer Res* 2001;61:5964–8.
33. Reker S, Becker JC, Svane IM, Ralfkiaer E, thor Straten P, Andersen MH. HLA-B35-restricted immune responses against survivin in cancer patients. *Int J Cancer* 2004;108:937–41.
34. Reker S, Meier A, Holten-Andersen L, et al. Identification of novel survivin-derived CTL epitopes. *Cancer Biol Ther* 2004;3:173–9.
35. Casati C, Dalerba P, Rivoltini L, et al. The apoptosis inhibitor protein survivin induces tumor-specific CD8(+) and CD4(+) T cells in colorectal cancer patients. *Cancer Res* 2003;63:4507–15.
36. Siegel S, Steinmann J, Schmitz N, Stuhlmann R, Dreger P, Zeis M. Identification of a survivin-derived peptide that induces HLA-A*0201-restricted antileukemia cytotoxic T lymphocytes. *Leukemia* 2004;18:2046–7.
37. Schmidt SM, Schag K, Muller MR, et al. Survivin is a shared tumor-associated antigen expressed in a broad variety of malignancies and recognized by specific cytotoxic T cells. *Blood* 2003;102:571–6.
38. Coughlin CM, Vance BA, Grupp SA, Vonderheide RH. RNA-transfected CD40-activated B cells induce functional T-cell responses against viral and tumor antigen targets: implications for pediatric immunotherapy. *Blood* 2004;103:2046–54.
39. Lee KH, Panelli MC, Kim CJ, et al. Functional dissociation between local and systemic immune response during anti-melanoma peptide vaccination. *J Immunol* 1998;161:4183–94.
40. thor Straten P, Guldborg P, Grønbo K, Zeuthen J, Becker JC. *In situ* T-cell responses against melanoma comprise high numbers of locally expanded T-cell clonotypes. *J Immunol* 1999;163:443–7.
41. Otto K, Andersen MH, Eggert AO, et al. Therapy-induced T cell responses against the universal tumor antigen survivin. *Vaccine* 2004;23:884–9.
42. Pisarev V, Yu B, Salup R, Sherman S, Altieri DC, Gabrilovich DI. Full-length dominant-negative survivin for cancer immunotherapy. *Clin Cancer Res* 2003;9:6523–33.
43. Hadrup SR, Gehl J, Sorensen RB, Geertsen PF, Straten PT, Andersen MH. Persistence of survivin specific T cells for seven years in a melanoma patient during complete remission. *Cancer Biol Ther* 2006;5:480–2.
44. Xiang R, Mizutani N, Luo Y, et al. A DNA vaccine targeting survivin combines apoptosis with suppression of angiogenesis in lung tumor eradication. *Cancer Res* 2005;65:553–61.
45. Wobser M, Keikavoussi P, Kunzmann V, Weininger M, Andersen MH, Becker JC. Complete remission of liver metastasis of pancreatic cancer under vaccination with a HLA-A2 restricted peptide derived from the universal tumor antigen survivin. *Cancer Immunol Immunother* 2006;55:1294–8.