Nuclear and Cytoplasmic Survivin: Molecular Mechanism, Prognostic, and Therapeutic Potential

Roland H. Stauber, Wolf Mann, and Shirley K. Knauer

Department of Otorhinolaryngology, Molecular and Cellular Oncology, University of Mainz, Mainz, Germany

Abstract
Survivin’s proposed dual role as an apoptosis inhibitor and a mitotic effector positioned it in the front line of cancer research. Notably, survivin is detected as a cytoplasmic and nuclear protein in cancer patients, which stimulated numerous studies to investigate and to speculate on the functional and prognostic significance of its dynamic localization. Recent evidence shows that the direct interaction of survivin with the nuclear export receptor Crm1 is critically involved in its intracellular localization and cancer-relevant functions. Here, we review our current understanding of the Crm1/survivin interface and discuss its potential prognostic and therapeutic relevance. [Cancer Res 2007;67(13):5999–6002]

Introduction
A failure in passing on the duplicated genetic material to both daughter cells together with resistance to apoptosis can contribute to cellular transformation and cancer progression. Survivin, as the smallest member of the inhibitor of apoptosis protein (IAP) family, seems to intersect both processes and thus has aroused keen interest in disparate areas of basic and translational research (refs. 1, 2 and references therein). Survivin is expressed during development and in proliferating cells, but largely undetectable in most differentiated tissues, in the absence of stress conditions. By contrast, survivin is highly expressed in liquid and solid tumors, and its expression has been correlated with resistance against cancer therapy–induced apoptosis and abbreviated patient survival (see ref. 1).

Besides its role as an IAP, survivin acts as a subunit of the chromosomal passenger complex (CPC; ref. 2) and as a regulator of microtubule dynamics (1). The CPC, composed of the Aurora-B kinase, Borealin, and INCENP, corrects attachment errors between chromosomes and the mitotic spindle, regulates the quality-control checkpoint, and ensures the correct completion of cytokinesis (2). The typical chromosomal passenger localization pattern of survivin can be observed not only in normal but also in tumor cells (Fig. 1A; refs. 2, 3). In normal cells, expression of survivin peaks at the G2-M transition of the cell cycle, whereas the cell cycle–dependent transcriptional control of survivin is deregulated by various oncopgenic pathways in cancer cells (see ref. 4). Hence, survivin is found in the majority of interphase tumor cells in patients (refs. 5, 6; Fig. 1B), which supports its bifunctional role. Moreover, the observation that survivin can be found not only in the cytoplasm but also in the nucleus of tumor and proliferating normal cells (e.g., endothelial and CD34+ stem cells; ref. 7) stimulated the hypothesis that these subcellular pools may coincide with different survivin functions (5). Nuclear survivin was suspected to control cell division, whereas cytoplasmic/mitochondrial survivin was considered cytoprotective. Consequently, the intracellular localization of survivin in tumor cells and its significance as a prognostic marker was analyzed in many patient-based studies, albeit with opposing conclusions (reviewed in ref. 5).

To rationally evaluate the significance and prognostic value of the dynamic localization of survivin, fresh evidence concerning its underlying molecular mechanism is now available.

The Role of Crm1 in Controlling Survivin Localization
In eukaryotic cells, an important mechanism to control the cellular localization of macromolecules is active nucleocytoplasmic transport. Nucleocytoplasmic transport takes place through the nuclear pore complex, is regulated by specific signals binding to transport receptors, and is active in all cell types, including tumor cells (8). Nuclear import is mediated by short stretches of basic amino acids, termed NLS, which interact with import receptors in the cytoplasm (8). Nuclear export signals (NESs) are leucine-rich, interact with the export receptor Crm1 in the nucleus, and depend on the small GTPase Ran, which controls the Crm1/substrate interaction (for details, see ref. 8). Regulated subcellular localization has been proposed for essential apoptosis and cell cycle regulators (refs. 9, 10 and references therein). For survivin, several studies reported the nuclear accumulation of cytoplasmic survivin upon treatment with leptomycin B (LMB), a drug that irreversibly blocks all NES/Crm1 interactions and thus nuclear export (refs. 3, 11; Fig. 1B). Consistent with these results, NES were identified in the linker region between survivin BIR domain and the COOH-terminal α helix (Fig. 1C; refs. 3, 12). In addition, the survivin splice variants survivin ΔBIR and survivin Δα, lacking the NES (Fig. 1C and D), do not localize predominantly to the cytoplasm (see ref. 13). The NES reported by Knauer et al. (refs. 3, 14; V89KQFEELTL107) confers the RanGTP-dependent Crm1 interaction in vitro and in vivo, is conserved in all mammalian survivin proteins, and matches the consensus sequence for leucine-rich NES (15). Colnaghi et al. (12) suggest amino acids 96 to 104 as the NES, based on the observation that specific mutations in this region (L96T/LGEFLKL104→ CTAGGEFLKL104) affected the cytoplasmic localization of survivin. However, this mutation did not completely abolish the interaction with Crm1 in vitro, indicating only partial inactivation of the survivin/Crm1 axis. Although these sequences partially overlap, further studies are required to unequivocally identify the NES in survivin. This is prerequisite to understanding the full effect of active transport on the biological activity of survivin and to investigating whether NES-inactivating mutations occur in cancer patients.
Figure 1. Dynamic intracellular localization of survivin and proposed model of how the survivin/Crm1 interface is required for the dual activity of survivin. A, centromeric survivin in a metaphase head and neck squamous cellular carcinoma (HNSCC) tumor cell is visualized by immunohistochemistry using an anti-survivin antibody. The centromeric localization of ectopically expressed survivin-GFP in the HNSCC cell line 1624 is affected by treatment with LMB or mutation of the NES (survivin ΔNES-GFP). Bar, 10 μm. Right, role of Crm1 in targeting the CPC to the centromere. Borealin is complexed with Survivin, which can bind to Aurora-B kinase and is incorporated into the CP holocomplex by interacting with INCENP. The NES in Survivin mediates recruitment of Crm1/RanGTP, which guides the CPC to the centromeres by a still unknown mechanism. This process might be catalyzed by the activity of the guanine nucleotide exchange factor RCC1 or TD60. Hydrolysis of RanGTP, by factors like RanBPs/Ran-GAP1, may facilitate the release of Crm1 and deposition of the CPC at the inner centromere. B, examples of cytoplasmic and nuclear staining of survivin in HNSCC tumor sections visualized by immunohistochemistry. Fluorescence microscopy shows that survivin-GFP localizes predominantly to the cytoplasm and accumulates in the nucleus upon LMB treatment or genetic inactivation of the survivin/Crm1 interaction (survivin ΔNES-GFP). Bar, 10 μm. Right, in interphase cells, nuclear export promotes a high cytoplasmic (and mitochondrial) concentration of survivin to counteract proapoptotic stimuli. C, domain organization of survivin and its splice variants. NES is indicated by the arrow. D, position of the NES within the NMR structure of wild-type (wt) survivin (PDB 1XOX). Ribbon representation of the backbone superposition (residues 1–117). Residues 89 to 98 encompassing the NES (green).
On the other hand, nuclear survivin in tumor cells is unlikely to be caused by active nuclear import because microinjection experiments revealed that survivin does not harbor an active NLS (3, 6). Thus, the low molecular weight of survivin (16,5 kDa) enables survivin to enter the nucleus by passive diffusion. Consistent with these data, no function of survivin depending on active nuclear import has been identified. Whether the reported nuclear accumulation of survivin upon irradiation or the potential function of survivin in post-irradiation DNA damage repair requires active transport remains to be investigated.

The economy of the eukaryotic cell is impressively underlined by the recent discovery that cellular components, regulating nucleocytoplasmic transport in interphase cells, are critically involved in controlling multiple mitotic processes (see ref. 16). These are accomplished by the Ran-GTPase system in conjunction with specific receptors of the importin-β family; in addition, Crm1 has been identified as an essential mitotic effector (16, 17). These findings help to understand the observation that LMB treatment not only resulted in nuclear accumulation of survivin in interphase cells but also affected the centromeric localization of survivin and of CPC components during mitosis (Fig. 1A and B; refs. 3, 11). Complete genetic inactivation of the survivin/Crm1 interaction by mutation of essential NES residues resulted in a protein (survivinΔNES) that not only resembled LMB-treated survivin in interphase cells but also failed to correctly localize during mitosis (ref. 3; Fig. 1A and B). Showing that NES-deficient survivin is still able to interact with the CPC components Aurora-B, Borealin, and INCENP (3) suggests that targeting of the CPC to the centromere but not CPC formation seems to require the Crm1/survivin interaction. Thus, Crm1 seems to be transiently involved in the transport of the CPC to the centromere, rather than in CPC anchoring (Fig. 1A), which clearly requires other CPC proteins (see refs. 2, 18).

The Survivin/Crm1 Interface Is a Determining Factor for the Bifunctional Role of Survivin

The significance of the Crm1/survivin interaction for the biological functions of survivin is supported by several lines of evidence. First, the aberrant localization of NES-deficient survivin during mitosis provides a molecular rationale for its failure to compensate for the loss of the endogenous protein and to rescue proper mitosis (3). By contrast, partial inactivation of the Crm1/survivin interface did not significantly affect survivin function as a mitotic effector in cancer cell lines (12). In addition, treatment of cancer cell lines with LMB resulted in mitotic defects and cell death (14), although LMB affects other proteins besides survivin. Second, although the molecular details (i.e., how survivin counteracts apoptosis) need further investigations (see refs. 1, 2), most of the mechanism would benefit from a high cytoplasmic concentration of survivin (ref. 14; Fig. 1B). As such, nuclear export may provide a continuous source for the pool of mitochondrial survivin, which is suggested to inhibit apoptosis via intermolecular cooperation with cofactors (reviewed in ref. 1). This assumption is supported by independent evidence showing that only export competent survivin was able to efficiently counteract chemotherapy- and radiotherapy-induced apoptosis (12, 14). Finally, tumor transplantation experiments indicated that the survivin/Crm1 axis is required for efficient tumor growth in vivo (19).

Prognostic Relevance of Nuclear and Cytoplasmic Survivin

The current data suggest that preferential cytoplasmic survivin represents “cytoprotective survivin” in tumor cells, whereas nuclear survivin may be indicative for “impaired survivin function.” Hence, one could propose a reduced tumor-protective survivin activity for patients with predominantly nuclear survivin in their tumors, which ultimately may also translate into a favorable prognosis. Preferential nuclear survivin was indeed found as a favorable predictor for various tumor types, although some reports consider nuclear survivin to be associated with poor survival (refs. 5, 6, 14 and references therein). This difference may be associated with the tumor types and/or the biopsies examined (pre-therapeutic versus post-therapeutic), or be due to the variable criteria used to classify a tumor as nuclear survivin or cytoplasmic survivin. As the balance between cytoplasmic and nuclear survivin in tumor cells may be considered as an indicator for “active survivin,” it is advisable to quantitate not only expression levels but also the relative intracellular localization of survivin [e.g., by applying an immunoreactive score (IRS) for cytoplasmic (IRS\textsubscript{cyt}) and nuclear (IRS\textsubscript{nuc}) survivin (6)]. This system allows to analyze the prognostic relevance of overall (IRS\textsubscript{cyt} + IRS\textsubscript{nuc}) and dynamic (IRS\textsubscript{cyt} - IRS\textsubscript{nuc}) survivin expression levels. Clearly, to evaluate and compare the prognostic value of dynamic survivin in future studies, standardization of immunohistochemical assays is mandatory.

The molecular details why survivin shows nuclear accumulation in some tumors in contrast to others has to be examined further. Nuclear localization of shuttle proteins can be induced by the competition for export factors (15) or by interfering with the nuclear transport machinery. In addition, mutations in the survivin NES or enhanced binding to nuclear components may account for the pronounced nuclear localization of survivin observed.

The Survivin/Crm1 Interface: a Potential Therapeutic Target?

Because the survivin “network,” intersecting essential cellular pathways, is exploited in virtually every cancer, survivin is vigorously pursued as a promising target for cancer therapy by various pharmacogenetic strategies (reviewed in ref. 4). As the evolutionary, conserved Crm1/survivin axis is essential for the dual activity of survivin (Fig. 1), it seems plausible that molecular decoys selectively targeting the survivin/Crm1 interaction will interfere with survivin function and may be of therapeutic relevance. Although unspecific export inhibitors (e.g., LMB) have been identified and also proposed for anticancer therapy, Crm1-directed inhibitors cannot be used in therapeutic applications due to their toxic side effects by blocking all Crm1-mediated transport (see ref. 10). Therefore, protein-specific transport inhibitors are urgently needed. Despite intense investigation, the detailed molecular mechanism regulating the orchestration and specificity of nuclear export is still not understood, but it may involve transient protein modifications (see ref. 10). Although survivin is subjected to various post-translational modifications, which affect protein stability (see refs. 1, 2), ubiquitination (20) or phosphorylation, as reported for neoplophosmin (17), seems not to regulate the survivin/Crm1 interaction (3). However, NES can be grouped into specific categories according to their activity in vivo (15). These differences represent an attractive opportunity to selectively block export and the biological functions of proteins by the generation of

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NES-specific inhibitors (14). The nuclear magnetic resonance structure of the survivin dimer (Fig. 1D), together with cell-based translocation assays (10), now encourage screens to identify NES-specific survivin antagonists. Pharmacologic targeting of the survivin/Crm1 interaction will most likely also affect the function of CPC proteins, including the mitotic kinase Aurora-B. Consequently, combining survivin-NES antagonists with Aurora-B kinase inhibitors currently in clinical trials may result in a more effective tumor therapy.

Conclusions
The recent insights into the functional significance of the survivin/Crm1 axis not only improved our understanding of the survivin “network” but also underscored the complexity of the mechanisms regulating cell division and cell survival. Survivin can shuttle between the nucleus and the cytoplasm, and the function of Crm1 as an export receptor creates a cytoplasmic survivin concentration gradient, which is counteracted by passive diffusion. The high cytoplasmic concentration of survivin may promote its cytoprotective function by facilitating survivin interplay with the apoptotic machinery in cancer cells. During mitosis, the Crm1/survivin interaction is critically involved in tethering the CPC to the centromeres and thus ensures proper chromosome segregation. In the future, it is imperative to determine whether a specific pharmacologic inhibition of the survivin/Crm1 interface can be achieved. To investigate whether such inhibitors show any undesired off target effects, and whether these drugs are effective in inhibiting the cancer-promoting functions of survivin in somatic cancer cells, tumor endothelial, and cancer stem cells, are the challenges for the future.

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References
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