The Role of Survivin for Radiation Therapy

Prognostic and Predictive Factor and Therapeutic Target

Gianni Capalbo[1], Claus Rödel[1], Roland H. Stauber[2], Shirley K. Knauer[2], Matthias Bache[3], Matthias Kappler[3], Franz Rödel[1]

Background: Survivin, the smallest member of the inhibitor of apoptosis protein (IAP) family, is a bifunctional protein that has been implicated in both control of cell division and inhibition of apoptosis.
Material and Methods: This review specially focuses on clinical and experimental data on the relevance of survivin in radiooncology and its role as a therapeutic target to radiosensitize tumor cells.
Results: As compared to normal tissue, survivin is overexpressed in tumors and appears to be closely related to tumor malignancy and treatment response. In addition, survivin is involved in the resistance of tumor cells to both chemotherapy and ionising irradiation. Due to these properties, survivin has been proposed as an attractive target for anticancer therapies. Several preclinical studies have demonstrated that suppression of survivin, by the use of antisense oligonucleotides, small interfering RNAs, ribozymes and the application of dominant negative mutants, increases apoptotosis, diminishes tumor cell survival and reduces tumor growth potential.
Conclusion: Survivin displays a relevant prognostic and predictive factor as well as a promising molecular target to improve the effectiveness of radiotherapy.

Key Words: Survivin · Apoptosis · Therapeutic target · Radiooncology

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Die Bedeutung Survivins für die Radiotherapie. Prognostischer und prädiktiver Faktor und therapeutischer Angriffspunkt

Hintergrund: Survivin, das kleinste Mitglied der „Inhibitor of Apoptosis Protein" (IAP)-Familie, ist ein bifunktionelles Protein mit essentiellen Funktionen in der Zellteilung und Hemmung der Apoptose.
Material und Methoden: Dieser Artikel konzentriert sich auf klinische und experimentelle Daten zur Relevanz von Survivin in der Radioonkologie sowie dessen Rolle als therapeutische Zielstruktur für eine Radiosensibilisierung von Tumorzellen.
Ergebnisse: Im Vergleich zu Normalgeweben wird Survivin in Tumoren überexprimiert und ist eng mit der Tumormalignität und dem Therapieansprechen assoziiert. Zudem ist Survivin an der Vermittlung einer Tumorzellresistenz gegenüber Chemotherapie und ionisierender Bestrahlung beteiligt. Aufgrund dieser Eigenschaften wird Survivin als eine attraktive Zielstruktur für die Krebstherapie angesehen. In mehreren präklinischen Untersuchungen konnte gezeigt werden, dass durch eine Antisense-Oligonukleotid-, siRNA- oder Ribozym-vermittelte Hemmung sowie nach Applikation dominant negativer Mutanten die Apoptoserate gesteigert wird. Die Überlebensrate der Krebszellen wie auch das Tumorwachstumspotential können dadurch gemindert werden.
Schlussfolgerung: Survivin zeigt hohe Relevanz als prognostischer und prädiktiver Marker und stellt einen vielversprechenden molekularen Angriffspunkt zur Verbesserung der Effektivität der Radiotherapie dar.

Schlüsselwörter: Survivin · Apoptose · Therapeutische Zielstruktur · Radioonkologie

Introduction

By disturbing the balance between proliferation and cell death, defects in the apoptosis pathway contribute to a variety of diseases including cancer [8, 21, 44]. Causing irreparable cellular damage triggering apoptosis is a key mechanism by which chemotherapy and ionizing radiation kill tumor cells [6, 20, 62]. Alterations in the expression of apoptosis-related proteins, like the inhibitor of apoptosis (IAP) protein family, however, influence the response to anti-cancer treatment. IAP gene amplification and increased protein expression oc-

[1]Department of Radiotherapy and Oncology, University of Frankfurt, Germany,
[2]Department of Otorhinolaryngology, Molecular and Cellular Oncology, University Hospital of Mainz, Germany.
[3]Department of Radiotherapy, University of Halle-Wittenberg, Germany.

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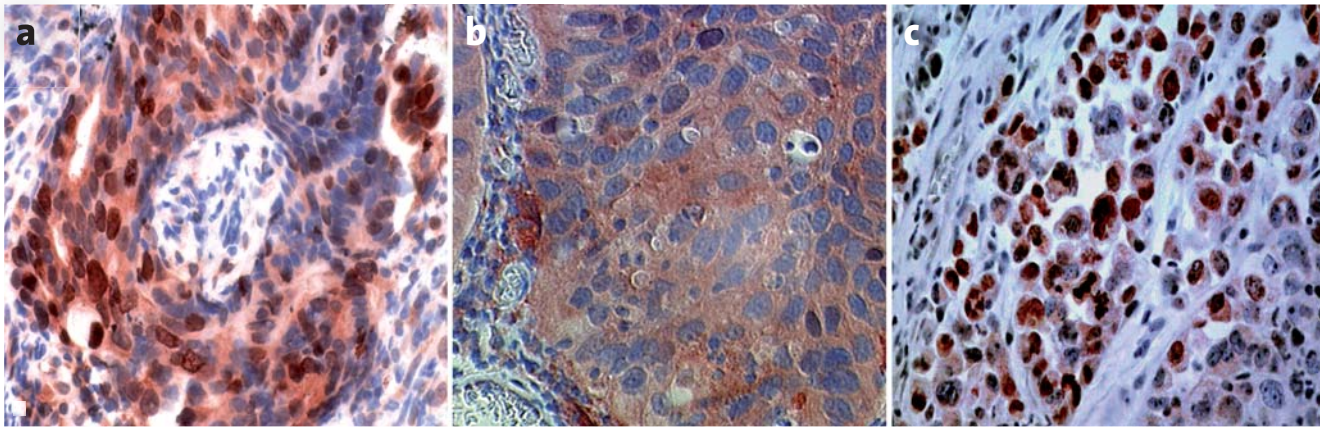

Figures 1a to 1c. Examples of intense immunohistochemical staining of survivin in tumor cells, but not in the surrounding normal tissue of patients with rectal (a), bladder (b) and head and neck (c) carcinoma with detection of the protein in the cytoplasm and the nucleus (original magnification x 40).

Abbildungen 1a bis 1c. Beispiele einer starken immunhistochemischen Anfärbung von Survivin in den Tumorzellen, nicht jedoch im umgebenden Normalgewebe von Patienten mit Rektum- (a), Blasen- (b) und Kopf-Halstumoren (c) mit nukleärer und zytoplasmatischer Anfärbung des Proteins (Vergrößerung 40fach).

curs in many cancers and displays a pivotal pathway by which cancer cells acquire resistance to therapeutic treatment [22, 54]. This review focuses on the role of survivin, the smallest member of the IAP-family with unique properties, as a prognostic and predictive marker and molecular target for innovative radiooncological treatment strategies.

Survivin Structure and Function

The human survivin gene, which is located at the telomeric region of the chromosome 17, encodes a 16,5 kD protein of 142 amino acids, organized as a stable homodimer [2]. Survivin is structurally composed of a single Baculovirus IAP Repeat (BIR) domain and a COOH-terminal α-helical coiled-coil domain. It does not contain a RING-finger domain, found in other IAP family members [35]. Consistent with the dual function survivin can shuttle between the nucleus and the cytoplasm and hence, can associate with different subcellular components [15, 58]. Alternative splicing of survivin pre-mRNA results in four survivin variants (survivin-ΔExon3, survivin-2B, survivin-2α, survivin-3B) with diverse cellular localization patterns and anti-apoptotic potential, which are however controversially discussed to inhibit or to promote wild type survivin's activity [5, 9, 30, 41]. Survivin shows a cell-cycle dependent expression with a marked increase in the G_2/M phase [34, 39]. The transcriptional control of survivin expression involves CDE/CHR (cell-cycle-dependent element/cell-cycle gene homology region) bipartite DNA elements that are located in the proximal survivin promoter [38]. A transcriptional repression of survivin is induced by wild-type p53 [63]. Also non-cell-cycle dependent transcriptional regulation of survivin has been described, which is mediated, amongst others, by Wnt/β-catenin/Tcf signalling [29]. Furthermore, the protein is phosphorylated by $p34^{cdc2}$/cyclin-B kinase resulting in an increased protein stability and accumulation at mitosis [45]. In addition, an association with Hsp90 causes stability of survivin by preventing it from proteasomal degradation [16].

Survivin acts as a subunit of the chromosomal passenger complex (CPC) composed of the mitotic kinase Aurora-B, Borealin/DasraB and the Inner Centromere Protein (INCENP) (reviewed in [36]). The CPC plays a key role in coordinating chromosome segregation with cytokinesis. In this context, survivin has been implicated in binding of the microtubules of the mitotic spindle, centromeres, kinetochores and intracellular midbodies, enabling coordinated cellular division [36, 60]. Inhibition of survivin expression or disruption of survivin interaction with microtubules induces defective cytokinesis with hyperploidy, multipolar mitotic spindles, and supernumerary centrosomes [12, 37].

Besides its role as a subunit of the CPC, survivin also acts as an inhibitor of apoptosis, probably preventing cell death by interfering with caspase activity or capture of inhibitors of other IAP family members [7, 14, 42, 56, 57]. However, the detailed molecular mechanisms by which survivin counteracts apoptosis are still not completely understood. Consistent with a high cytoplasmic concentration of survivin, a CRM1- (chromosome region maintenance 1) mediated pathway of nuclear export has been demonstrated. Interestingly the export seems to be required for the anti-apoptotic activity of survivin, as export-deficient survivin mutants failed to protect tumor cells against chemo- and radiotherapy-induced apoptosis [13, 31, 32, 53].

Survivin Expression in Normal and Tumor Tissues

Survivin expression in normal tissues is developmentally regulated and has been reported to be low in most terminally differentiated tissues [2]. However, recent studies have demon-

Table 1. Prognostic relevance of survivin expression in treatment response towards radiotherapy (RT) and radiochemotherapy.
Tabelle 1. Prognostische Bedeutung der Survivin-Expression für das Ansprechen auf eine Strahlentherapie und Radiochemotherapie.

Tumor	Patients (n)	Treatment	Survivin	Clinical autcome	References
Rectal cancer	54	Preoperative radiochemotherapy	Histochemistry	Inverse correlation with apoptosis, high survivin associated with worse local tumor control and survival	[49]
Rectal cancer	98	57 surgery alone, 41 preoperative short-term radiotherapy	Histochemistry	Related to worse survival both for patients treated with surgery alone and preoperative radiotherapy + surgery	[33]
Cervical carcinoma	44	Combined external radiotherapy and brachytherapy	Histochemistry	Correlated to poor overall survival, inverse correlation with hemoglobin	[4]
Cervical carcinoma	72	Combined external RT and brachytherapy	Histochemistry	Cytoplasmatic expression predicted local control	[59]
Naso-pharyngeal carcinoma	18	4 radiotherapy 14 radiochemotherapy	Affymetrix HGU133 array	Inverse correlation with apoptosis	[61]
Esophageal cancer	51	Preoperative radiochemotherapy	Real time PCR	No correlation with histomorphologic tumor regression, high survivin associated with superior survival rates	[64]
Oral squamous cell carcinoma	296	121 surgery alone 147 radiotherapy +/– chemotherapy	Histochemistry	High survivin associated with increased overall survival	[17]
Naso-pharyngeal carcinoma	80	Radiotherapy	Histochemistry	High or low nuclear survivin associated with worse clinical outcome as compared to intermediate survivin	[65]

strated survivin expression in a variety of normal adult cells and tissues like basal colonic epithelium, endothelial cells and hematopoietic progenitor cells [18, 19, 43]. Although there is growing evidence suggesting an emerging role of survivin in non-cancerous tissues, it has been consistently demonstrated that survivin is overexpressed in the majority of solid (Figure 1) and liquid human tumors. High expression of survivin is commonly associated with an enhanced proliferative index, reduced levels of apoptosis and increased rate of tumor recurrence. Furthermore, elevated levels of survivin and also predominantly cytoplasmic survivin have been associated with aggressive clinicopathologic features and show a strong correlation with reduced disease-free and overall survival rates in most studies (reviewed in [1, 58]).

Survivin as a Predictive Factor for Treatment Response towards Radiotherapy and Radiochemotherapy

Although the role of survivin as a prognostic marker is consistent within the literature, its value as a marker for the prediction of treatment response towards radiotherapy or radiochemotherapy is controversial (Table 1). In most retrospective trials, cancer patients with a high level of survivin expression exhibited increased resistance to radiotherapy. An analysis of 54 rectal cancer patients homogeneously treated with a combination of preoperative radiotherapy and 5-fluorouracil within the German Rectal Cancer Trial (CAO/ARO/AIO-94) revealed that survivin expression on pretreatment biopsies was inversely correlated with the level of spontaneous apoptosis. Moreover, high survivin expression was significantly associated with the risk of local tumor recurrences [49]. Knutsen et al. examined survivin expression in 98 rectal cancer patients who participated in the Dutch-TME- (total mesorectal excision) trial of preoperative short-course radiotherapy plus TME-surgery versus TME -surgery alone (57 underwent surgery alone, 41 received preoperative radiotherapy): Survivin positivity was independently related to inferior survival irrespective of treatment with or without radiotherapy [33].

Comparable results were obtained in another study, where the expression of survivin in biopsies of 44 patients with cervical cancers treated with definitive radiotherapy was determined by immunochemistry. A high survivin expression was correlated to worse overall survival [4]. As recent findings have shown that tumor-specific expression of survivin is increased by hypoxia [64], the expression of survivin was investigated for its relationship to hypoxia parameters like hemoglobin level and Hif-1α (hypoxia-inducible factor) expression. In this context, an inverse correlation with the hemoglobin level and an association with expression of Hif-1α could be observed [4]. In another study, gene expression profiling on primary nasopharyngeal carcinoma (NPC) patients treated with radiotherapy alone (4 patients) or combined with chemotherapy (14 patients) was performed. The data demonstrated a central role of upregulated NF-κB (nuclear factor kappa B) and survivin in increasing resistance to apoptosis. Additionally, the role of survivin in resisting apoptosis in NPC was confirmed by RNA interference, demonstrating a distinct increase in apoptosis following irradiation with 6 Gy [55]. A correlation between survivin expression and locoregional control was further confirmed in tissue sections from 72 patients with cervical squamous cell carcinoma treated with radiation therapy. Interestingly, a relation between nuclear and cytoplasmic expression of survivin was noted in terms of local control. It

was shown, that cytoplasmic survivin expression correlated with an inferior local control rate [59].

In contrast to the studies mentioned above, Warnecke-Eberz et al. examined the suitability of survivin mRNA levels to predict histopathologic tumor response following neoadjuvant radiochemotherapy for 51 patients with locally-advanced esophageal cancer. In this study, survivin expression on pretreatment biopsies did not predict the extent of histomorphologic tumor regression following preoperative therapy. In addition, high levels of survivin mRNA were intriguingly associated with superior survival rates [61]. Freier et al. evaluated survivin expression using a tumor tissue microarray derived from 296 patients with advanced primary oral squamous cell carcinoma. Within the subgroup of patients, who received radiation therapy (n = 147), high survivin expression was reported to be the only predictor of favourable overall survival [17]. To determine the prognostic significance of key molecular factors in NPC, biopsies from 80 patients treated with curative radiation were evaluated for the expression of EBV (Epstein-Barr virus) as well as p53, Bcl-2 and survivin. It was shown that p53, Bcl-2 and nuclear survivin are overexpressed proteins in EBV-positive NPC. Patients with a low or high proportional expression level of nuclear survivin fared worse than patients with intermediate survivin expression [65].

Survivin as Determinant of Radiation Response – in vitro Results

Considering the major role of programmed cell death following irradiation, survivin was consequently supposed to be a radioresistance factor in tumor cells. In a panel of established pancreatic cancer cell lines, Asanuma et al. were the first to describe an inverse relationship between survivin mRNA expression and sensitivity to ionizing radiation. In addition, survivin expression was increased by sublethal doses of irradiation, suggesting a function as an inducible radioresistance factor [3]. Rödel et al. showed an inverse correlation between survivin expression and apoptotic response to irradiation. Comparison of colorectal carcinoma cell lines of low (SW480) and high (SW48) radiosensitivity showed a higher constitutive expression of survivin in SW480 cells with a pronounced induction of survivin after irradiation. In contrast, expression of survivin was low and not induced in radiosensitive SW48 cells after irradiation [48]. Chakravarti et al. also demonstrated that survivin expression is much higher in two radioresistant glioblastoma multiformi cell lines (GM20 and GM21) as compared with two radiation sensitive cell lines (GM22 and GM23). Additionally, a pan-cell cycle expression of survivin in the radioresistant GM20 and GM21 cell lines was observed upon radiation exposure [11].

Interestingly, it was shown that survivin is transcriptionally down-regulated by radiation in normal human endothelial cells independently of cell cycle, but that this mechanism is defective in various cancer cell lines [35]. To explore the mechanism by which survivin is down-regulated, reporter gene assays were performed after radiation, suggesting a decrease in survivin promoter activity. However, the observed transcriptional repression of survivin upon irradiation was not mediated through the tumor suppressor p53. In addition, the authors demonstrated that the combination of survivin inhibition and irradiation resulted in significantly decreased cell survival of cancer cells, concluding that survivin overexpression leads to radiotherapy resistance [40].

To investigate the relationship between p53 and survivin regarding radioresistance, two sarcoma cell lines, A-204 with wild-type p53 and US 8-93 with mutated p53, were treated with a combination of survivin siRNA and irradiation. Suppression of survivin caused radiosensitization in the wt-p53 cell line A-204, whereas no effects on radiosensitivity were observed in the cell line US 8-93 (mt-p53). Furthermore, activity of caspases 3 and 7 was increased after a combined treatment of siRNA and irradiation only in the wt-p53 cell line, suggesting that the wt-p53-caspase pathway is of importance for the radiosensitization induced by targeting survivin [25, 26]. Furthermore, treatment with survivin isoform specific siRNA (wild-type, survivin-2B, delta 3) reduced clonogenic survival under normoxic conditions as well as under hypoxic conditions [27]. However, whereas overexpression of wildtype and survivin-3B protected cells against irradiation, overexpression of the other survivin isoforms had not effect on radiosensitivity [30].

Survivin as a Therapeutic Target for Radiation Sensitization

Overall, the results obtained in a variety of studies indicate survivin to be an important factor in determining the radiation response of human tumor cells. Due to its differential expression in cancerous tissues and its potential requirement for maintaining cancer-cell viability [1], survivin displays a suitable molecular target for radiosensitization. Different strategies to counteract survivin's expression or activation in tumor cells have demonstrated that its inhibition resulted in an increase in spontaneous and radiation-induced apoptosis and thus enhances the response towards cancer treatments including chemotherapy and radiotherapy [47, 58], These strategies comprise antisense oligonucleotides, ribozymes, siRNA and dominant-negative mutation [10, 11, 46, 52]. Due to the fact that CDC2 phosphorylation of survivin is required for cancer-cell viability, the use of kinase inhibitors, including CDC2 antagonists, is another promising approach to enhance the response towards cancer treatments [23].

Cao et al. examined the effects of radiosensitization in the H460 lung cancer model via suppression of survivin and XIAP (X-linked mammalian inhibitor of apoptosis protein). Application of antisense oligonucleotides (ASOs) directed against survivin or XIAP reduced survival of H460 cells in culture and increased tumor growth delay of H460 xenografts when combined with radiation [10]. Similar results were reported in the pancreatic cancer cell line AsPC-1. Radiation increased survivin promotor activity in AsPC-1 cells resulting in strength-

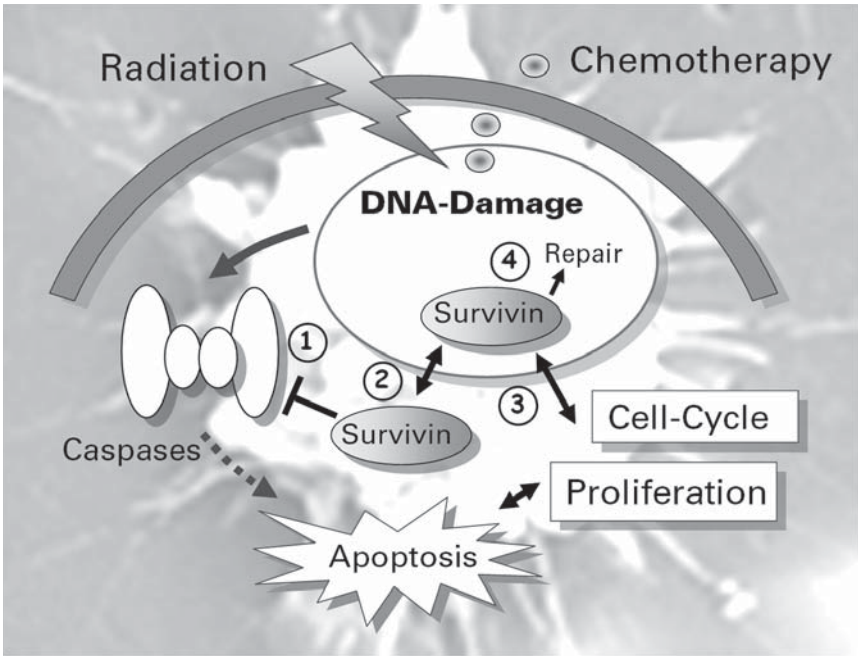

Figure 2. Schematic presentation of the role of survivin as a radioresistance factor and molecular target for radiosensitization of tumor cells. (1) Enhanced expression of survivin mediates radiation resistance of tumor cells through suppression of apoptosis by interfering with caspase activity [3, 11, 26]. (2) The anti-apoptotic activity of survivin is dependent on a CRM1-mediated pathway of nuclear export, as export-deficient survivin mutants failed to protect tumor cells against radiation-induced apoptosis [58]. (3) Besides its role as an inhibitor of apoptosis, survivin also acts as a cell cycle regulatory protein, enabling coordinated cellular division. Accordingly, depletion of survivin alters cell cycle distribution, resulting in a G2 and mitotic arrest [25, 50]. (4) In addition, survivin appears to be involved in the regulation of DNA repair, thereby enhancing tumor cell survival upon radiation exposure [11, 50].

Abbildung 2. Schematische Darstellung der Funktion von Survivin als Radioresistenzfaktor und molekulare Zielstruktur einer Strahlensensibilsierung von Tumorzellen. 1: Die Überexpression von Survivin in Tumorzellen führt zu einer Strahlenresistenz, die durch Hemmung der Caspase-Aktivität und somit der Apoptose vermittelt wird [3, 11, 26]. 2: Die anti-apoptotische Aktivität von Survivin hängt dabei von einem CRM1-abhängigen Kernexport ab, da Export-defiziente Survivin-Mutanten nicht in der Lage sind, Tumorzellen gegen strahleninduzierte Apoptose zu schützen [58]. 3: Neben der Funktion als Apoptose-Inhibitor stellt Survivin auch ein Zellzyklus regulierendes Protein dar, welches eine koordinierte Zellteilung ermöglicht. Dementsprechend führt die Hemmung von Survivin zu einer veränderten Zellzyklusverteilung und einem vermehrten G2- und mitotischen Arrest [25, 50]. 4: Darüber hinaus scheint Survivin an der Regulation von DNA-Reparaturprozessen beteiligt zu sein und auch auf diese Weise das Tumorzell-Überleben nach Bestrahlung zu beeinflussen [11, 50].

ened expression of survivin. Attenuation of survivin expression by siRNA treatment diminished the radioresistance of AsPC-1 cells by augmenting caspase-3 activity upon radiation [24]. Equally, the siRNA-mediated suppression of survivin in SW480 and HCT15 colorectal cancer cells increased apoptosis and caspase 3/7 activity after irradiation, which resulted in decreased cell viability and clonogenic survival. Furthermore, a higher incidence of DNA double-strand breaks after irradiation was observed, as indicated by a higher amount of Ser^{139}-phoshorylated histone γH2AX staining after survivin siRNA treatment [50, 51]. Using adenoviral vectors containing a dominant-negative survivin construct, Chakravarti et al. also reported a decreased DNA repair capacity of glioblastoma cells upon radiation exposure as measured by a comet assay [11]. Thus, the radiosensitizing activity of survivin inhibition seems to be multifaceted and involves caspase-dependent and caspase-independent mechanisms like impaired DNA repair as well as an altered cell-cycle distribution, formation of multinucleated cells and mitotic arrest and subsequent cell death (Figure 2) [25, 28].

Conclusion

Results from a variety of preclinical studies using different strategies to interfere with survivin function provided direct evidence that targeting the protein inhibits tumor growth and increases irradiation-induced cell-kill. Additionally, due to its prognostic relevance and predominant expression in tumor cells, survivin has been proposed as a promising molecular target for cancer therapy. The applicability of survivin-driven strategies for the clinical practice is currently under investigation as the first survivin inhibitors recently entered phase I/II trials. Although these clinical trials do not include radiotherapeutic approaches at present, survivin inhibitors may represent a novel type of molecular antagonists to improve the effectiveness of radiotherapy/radiochemotherapy in patients with high survivin expression.

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Address for Correspondence
Gianni Capalbo, PhD
Department of Radiotherapy and Oncology
University of Frankfurt
Theodor-Stern-Kai 7
60590 Frankfurt am Main
Germany
Phone: (+49/69) 6301-6637, Fax: -5091
e-mail: gianni.capalbo@kgu.de