Investigations of survivin: the past, present and future

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1. ABSTRACT
Survivin is a member of the inhibitors-of-apoptosis protein (IAPs) family. It promotes cell survival through interference with multiple cell cycle-related proteins such as INCENP and Aurora B kinase. Survivin also inhibits cell death through interference with both caspase-dependent and -independent cell apoptosis. Interestingly, recent evidence suggests that survivin may also play a role in the regulation of cancer cell autophagy. At the clinical level, studies on clinical specimens have shown that survivin expression is up-regulated in various human cancers and its up-regulation is associated with tumour resistance to both chemotherapy and radiation therapy. On the basis of these findings, survivin has been proposed as an attractive target for new anti-cancer interventions. However, despite the role that survivin plays in cancer cell survival and anti-drug response, the development of survivin inhibitors is relatively slow as compared to other therapeutic inhibitors for cancer treatment. In this review, the relationships between survivin expression and the causation of drug resistance in cancers are re-addressed. This review also summarizes the recent development of survivin inhibitors for clinical usage.

2. INTRODUCTION
Members of the inhibitor of apoptosis protein (IAP) family are important for inhibiting caspase activity and cell death in response to apoptotic stimuli. Eight IAPs are identified in humans: Apollon (Bruce) (1), cIAP-1 (HIAP-2/MIHB) (2), cIAP-2 (HIAP-1/MIHC) (3), ILP-2 (Ts-IAP) (4), NIAP (5), survivin (TIAP) (6), XIAP (ILP-1/MIHA) (7) and the recently identified livin (alternatively called ML-IAP or KIAP) (8). They are characterized by the possession of one or more baculoviral IAP repeat (BIR) domains of ~70 amino acid residues and structure-function studies of IAPs have demonstrated that IAPs require at least one BIR domain in order to suppress cell apoptosis. Although IAPs are widely expressed in normal tissues, their expression is significantly increased in various tumour tissues (9-13). The only exception is survivin, which is primarily expressed in fetal and cancerous tissues, but not in normal, developed adult tissues (14). Originally, survivin was thought to be able to inhibit caspase-dependent apoptosis and at the same time promote cell division by interfering with Aurora-B kinase. However, recent literature reveals that survivin plays multiple roles in the process of tumorigenesis and the causation of drug-
resistance. In this review, the underlying mechanism of survivin function is discussed. Furthermore, the relationships between survivin expression and the causation of drug resistance in cancers are re-addressed. This review also summarizes the recent development of survivin inhibitors for clinical usage.

3. SURVIVIN

3.1. The expression of survivin in human cancers

Survivin is a member of the IAP family, which is expressed during embryonic and fetal development. In fact, it has been demonstrated that survivin plays essential roles in both neurogenesis and hematopoiesis (15, 16). Unlike other IAPs, survivin is also expressed in various cancers, but not in differentiated normal tissue. In clinical situations, survivin was found to be expressed in various oral cancers. A study from Lin et al. in Taiwan has observed the expression of survivin in 97% (60/62) of oral epithelial dysplasia specimens (17). In addition, 98% (94/96) of oral squamous cell carcinoma specimens showed increased survivin expression (17). In contrast, the expression of survivin was not observed in adjacent normal oral mucosal tissues (17). Another study from Hsu et al., also in Taiwan, demonstrated that survivin was over-expressed in esophageal squamous cell carcinoma (18). In this study, the percentage of survivin expression in well differentiated (n=7), moderately differentiated (n=15) and poorly differentiated (n=24) esophageal squamous carcinomas was 60%, 74% and 80% respectively. In contrast, only 25% of normal esophageal epithelia specimens (n=8) expressed survivin (18). In addition, high survivin expression level was significantly associated with a shorter survival period (18). Another study performed by Preuss et al. revealed a correlation between survivin expression and survival period in patients with oropharyngeal cell carcinoma (19). In this study, patients with higher cytoplasmic survivin expression levels were shown to have a lower 5-year disease-free survival rate (19).

Besides being overexpressed in oral cancers, survivin was found to be over-expressed in patients with colorectal carcinoma and lymphoma (20). Two studies revealed that expression of survivin was significantly higher in adenomatous polyps and adenocarcinoma as compared to normal colorectal mucosa (21, 22). Survivin expression was also correlated with overall survival in patients with stage II colorectal carcinoma in the above independent studies (21, 22). On the other hand, survivin seems to play an important role in the transition of adenoma with low dysplasia to high dysplasia during human colorectal tumorigenesis (23). In one study, Kawasaki et al. demonstrated that only two percent of cases with adenoma with low dysplasia (n=171) were positive for survivin expression. In comparison, more than 50% of cases with adenoma with high dysplasia (n=42) and with carcinoma (n=60) were positive for survivin expression (23). Therefore, survivin over-expression seems to play important roles in the pathogenesis and the progression of some cancers.

3.2. Molecular functions of survivin

Since survivin is over-expressed in different cancers under clinical situations, various studies have been carried out to determine its underlying molecular mechanisms. At the molecular level, survivin is a bi-functional protein that acts as a suppressor of apoptosis and plays a central role in cell division (Figure 1). It has been suggested that survivin, possibly the mitochondrial fraction instead of the cytosol fraction, inhibits apoptosis through interference with caspases (24-26). A study using surface plasmon resonance spectroscopy showed that survivin directly binds to caspase-3 and caspase-7 with nanomolar affinity (24). In addition, myc-tagged survivin bound caspase-3 and caspase-7 in immunoprecipitation studies (24). It is not surprising that survivin is able to interfere with the activity of caspases, given that survivin contains a single baculoviral IAP repeat (BIR) domain and that the BIR domain was shown to be important in targeting caspases in various IAP family members (27). Interestingly, recent evidence indicates that survivin may also interfere with caspase-independent apoptosis. For example, it has been demonstrated that survivin interferes with the translocation of the apoptosis-inducing-factor (AIF). AIF is a flavoprotein that is normally confined to the mitochondrial intermembrane space, but induces chromatin condensation and fragmentation of DNA into high molecular weight forms of >50 kb when it translocates to the nucleus (28, 29). Interestingly, down-regulation of survivin induces the translocation of AIF in various cancer cell lines (30-32).

Besides interfering with both caspase-dependent and -independent apoptosis, survivin also promotes cell survival through interference with cell cycle-related kinases and microtubule networks (Figure 1). Survivin appears to function as a subunit of the chromosomal passenger complex (CPC) for the regulation of cell division. It has been demonstrated that survivin binds to the Aurora-B kinase, Borealin and INCENP (33-36). During mitosis, survivin locates to centromeres on a para-polar axis during prophase/metaphase, relocates to the spindle midzone during anaphase/telephase and disappears at the end of telophase (37). Over-expression of survivin has been shown to reduce centroosomal microtubule nucleation and suppress both microtubule dynamics instability in mitotic spindles and bidirectional growth of microtubules in midbodies during cytokinesis (38). In addition, it has been shown that intracellular loading of a polyclonal antibody to survivin induced microtubule defects and resulted in the formation of multipolar mitotic spindles (39).

Recently, it has been suggested that survivin may also play a role in the regulation of cancer cell autophagy (Figure 1). Results from Roca et al.'s study indicated that CCL2 (Chemokine (C-C motif) ligand 2, MCP-1, monocyte chemo-attractant protein-1) protects human prostate cancer PC3 cells from autophagic cell death via the phosphatidylinositol 3-kinase/Akt/survivin pathway (40, 41). Taken together, survivin appears to be a master molecule that regulates multiple survival pathways in cancer cells.
3.3. Survivin and drug resistance

Considering the direct interactions between survivin and various apoptotic/mitotic-related molecules, over-expression of survivin should enhance cancer cell survival and anti-drug ability under chemotherapeutic stress. In fact, over-expression of survivin has been connected to the causation of cancer-drug resistance in various studies (42-44). It has been demonstrated that the inhibition of apoptosis mediated by survivin contributed to cisplatin-resistance in lung cancers and possibly in gastric cancers (45, 46). Survivin expression was shown to be up-regulated in the gastric cancer cell line, MKN-45, in response to cisplatin treatment in vitro. In addition, the level of resistance was correlated to the amount of survivin over-expressed in the treated cells (46). On the other hand, targeting survivin with shRNA induces caspase-dependent apoptosis and enhances cisplatin sensitivity in squamous cell carcinoma of the tongue (47). Expression of survivin also interferes with the sensitivity to various anti-mitotic compounds and pro-apoptotic chemokines in cancer cells. Zaffaroni et al. showed that stable transfection of human ovarian carcinoma cells with survivin cDNA caused a four to six-fold increase in cell resistance to taxotere and taxol (48). A recent study from our laboratory also suggests that the over-expression of survivin counteracts the therapeutic effect of anti-mitotic compounds (microtubule destabilizing agents) BP0L075 and colchicine possibly through the stabilization of tubulin polymers in human oral cancer cells (30).

On the other hand, survivin may play a role in tumor cell resistance to TRAIL-induced apoptosis through cell cycle regulation. Transfection of a survivin anti-sense construct enhanced the sensitivity to TRAIL in human hepatocellular carcinoma both in vitro and in vivo (49). Survivin also plays an important role in inducing therapeutic resistance to endocrine therapy in both breast and prostate cancers. Tamoxifen has been widely used in endocrine therapy for estrogen receptor-positive breast cancer. A study demonstrated that targeting survivin by siRNA enhanced tamoxifen-induced apoptosis in MCF-7 breast cancer cells in vitro (50). In addition, over-expression of survivin is able to mediate resistance to anti-androgen therapy in human prostate cancer cells possibly through the inhibition of apoptosis (51).

Interestingly, a few reports suggest that the over-expression of survivin may induce drug-resistance through indirect mechanisms. It has been demonstrated that the over-expression of survivin induced the up-regulation of multi-drug resistance P-glycoprotein (Pgp) and subsequently reduced drug accumulation in MCF-7 breast cancer cells in vitro (52). Thus, survivin may modulate the turnover of Pgp or transport by Pgp in cells, resulting in anti-apoptosis and drug resistance. Furthermore, growing evidence indicates that transient up-regulation of survivin by VEGF and bFGF in normal endothelial cells of blood vessels is partly responsible for tumour angiogenesis and tumour resistance against chemotherapeutic drugs (53, 54). Up-regulation of survivin is also able to counteract the pro-apoptotic effect induces by TNF-α in blood vessel endothelial cells (55). Interestingly, a recent report suggests that the translocation of survivin into the nucleus may enhance DNA double strand breaks (DBD) repair capability in radiation-treated oral cancer cells by up-regulating the molecular sensor of DNA damage, Ku70 (56). This observation is indeed important as it indicates that besides the over-expression of survivin, the translocation of survivin into the nucleus may also play an important role in the interference with therapeutic efficiency in cancers.

3.4. Targeting survivin by anti-sense, siRNA and dominant-negative constructs

In the past decade, most targeted-therapies did not show great therapeutic advantages over traditional chemotherapies in clinical situations. This is due to the fact that cancer cells acquire anti-apoptotic properties through up-regulation or alteration of various pro-survival mechanisms simultaneously and targeting a single pathway by targeted-therapy is insufficient to induce cancer cell death. Furthermore, cancer cells are known to acquire drug resistance properties after prolonged treatment through both drug-target mutations and changes in the intracellular signaling pathways. Since survivin interferes with various cancer-related pathways, targeting survivin may intersect multiple cancer survival pathways simultaneously (instead of a singularly targeted pathway) and may give a better
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therapeutic outcome as compared to other targeted-therapies in clinical situations.

Under laboratory conditions, it has been widely demonstrated that targeting survivin induces cancer cell death and restores drug sensitivity to different chemotherapeutic compounds. Down-regulation of survivin by liposomal/adenoviral-delivered siRNA has been shown effective in inducing cell death of various cancers such as gastric carcinoma (57), esophageal carcinoma (58), renal clear cell carcinoma (59), bladder cancer (60) and cervical cancer (61). Interestingly, a recent study demonstrates that the delivery of survivin siRNA using a micro-bubble contrast agent combined with ultrasound exposure can effectively inhibit survivin expression and induce apoptosis in ovarian carcinoma cells (62). Targeting survivin by an anti-sense oligonucleotide was also successful in inducing cancer cell death. SPC3042 is a 16-mer locked nucleic acid (LNA) oligonucleotide that targets the expression of survivin. A pre-clinical study revealed that SPC3042 was able to reduce the amount of survivin protein present in PC3 prostate cancer cells and subsequently induce cell apoptosis (63). The same study also revealed that SPC3042 is capable of sensitizing prostate cancer cells to taxol treatment in vitro (63). On the other hand, targeting survivin by gene transfection of a dominant-negative construct (either T34A or C84A mutant) is able to induce the apoptosis of melanoma (64), lymphoma (65) and prostate cancer cells (66) in vitro and in vivo. The C84A dominant-negative mutant form of survivin was constructed on the basis of the finding that mutation of Cys84 to Ala in the extreme C-terminal region of the BIR domain disrupts the Zn²⁺ coordination sphere and subsequently abrogates survivin’s ability to inhibit apoptosis. On the other hand, phosphorylation of survivin on Thr34 is critical for the stability of survivin, and hence for its role in promoting cell cycle progression and caspase inhibition (67). Mutation of Thr34 to Ala completely abolished the phosphorylation of survivin, resulting in the dissociation of the survivin-caspase-9 complex (67). Recently, it has been demonstrated that proteins fused with cell-permeable domains such as TAT and R9 are able to penetrate cells without the use of transfection reagents. Targeting survivin by a recombinant cell-permeable (TAT or R9-tagged) dominant-negative survivin (T34A or C84A) protein was also shown to be effective in inducing cancer cell death as compared to traditional gene therapy (68, 69). Interestingly, Khan et al. reveals that a T34A dominant-negative mutant of survivin, without coupling to a cell-permeable domain or the use of transfection reagent, was able to be taken up by HeLa cells and subsequently induced caspase-dependent apoptosis (70). It is widely believed that cancer cells over-express survivin endogenously. Then the endogenously expressed survivin interferes with various intracellular survival-related molecules within the cell. However, results of Khan et al.’s study indicate that cancer cells may be capable of taking up survivin that is located in the extracellular space and subsequently promote the ability of cell survival.

3.5 Recent developments of the survivin-specific small molecule inhibitors

Despite previous success in the use of both anti-sense, siRNA and dominant-negative constructs to target survivin, the development of survivin-specific pharmacological (small molecule) inhibitors is relatively slow as compared to other cancer-related kinase inhibitors. For example, more than twenty aurora kinase-specific inhibitors have been developed for cancer treatment and more than six of such inhibitors have undergone various clinical trials in the last five years (71, 72). In contrast, only a few small molecule survivin inhibitors have been developed in the past ten years and only one survivin inhibitor, YM155, has successfully reached stage II clinical trials (63, 73-78). YM155 (1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro -1H-naphtho[2,3-d]imidazolium bromide) is an inhibitor that functions in inhibiting the transcription of survivin in cells (78). In pre-clinical models, the application of YM155 was shown to be effective in inducing apoptosis in various types of cancers such as prostate cancer, lung cancer and lymphoma (74, 77, 78). It has been demonstrated that YM155 induces the regression of human hormone-refractory prostate tumor in vivo (78). In a PC-3 prostate tumor xenograft animal model, a weekly three-day continuous infusion schedule of YM155 at dosages of 3 and 10 mg/kg inhibited tumor growth and induced massive tumor regression. In addition, administration of YM155 at dosages of 1 and 5 mg/kg induced 47% and 80% inhibition of tumor growth, respectively, in PC-3 orthotopic xenografts (78). YM155 was also shown to be able to sensitize human non-small cell lung cancer (NSCLC) cells to both platinum compounds and radiotherapy in vitro and in vivo (77, 79). The pharmacokinetics and the maximum tolerance dose (MTD) of YM155 have been evaluated in patients with non-Hodgkin’s lymphoma, docetaxel-refractory prostate cancer and non-small cell lung cancer. Phase I clinical trials did not reveal any significant toxicity related to the use of YM155 as a single agent (73, 75, 76). In studies by Tolcher et al. and Satoh et al., patients were treated with YM155 on a schedule of 168-hour continuous infusion every 3 weeks and the maximal tolerated dose (MTD) was defined as 4.8 and 8.0 mg/m²/d, respectively (73, 76). The non-hematological adverse events observed in these patients included stomatitis, pyrexia, nausea, arthralgia, urine microalbumin present, injection-site phlebitis, abnormal liver function test and decreased serum albumin level. Most adverse events were classified as Grade 1/2, with only one patient developing Grade 3 adverse effect (abnormal liver function). Increased level of serum creatinine was also noted as dose-limiting toxicity (DLT) in both trials, occurring only in the highest dose level. In Tolcher et al.’s trial, image responses occurred in three patients with non-Hodgkin’s lymphoma, while two patients with heavily-treated prostate cancer experienced prostate-specific antigen response. One patient with non-small cell lung cancer had a minor response to the YM155 treatment. In addition, nine patients achieved stable disease in Satoh et al.’s trial.

Even though various pre-clinical and phase I clinical studies indicated that YM155 could be an effective anti-cancer reagent, phase II clinical trials showed disappointing results. Giaccone et al.’s study revealed that
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the objective tumor response rate (ORR) to YM155 treatment (168-hour continuous infusion at a dose of 4.8 mg/m2/d) was approximately 5% in patient with advanced refractory non-small-cell lung carcinoma (75). In addition, around 70% of patients discontinued the regimen because of adverse effects (AE). In another study conducted by Lewis et al., similar administration schedule was prescribed for melanoma chemotherapy-naive patients (80). The result of ORR in this study was even more disappointing: the response rate was approximately 3%. Notably, four patients experienced severe adverse events (SAE) that were possibly related to the drug. The trial has also focused on the determination of YM155-related cardio-toxicity. In this study, four subjects had been reported to exhibit cardiac rhythm AEs. This issue is not yet confirmed to be drug-related, but it should be noted in future studies.

Besides direct survivin inhibitors, indirect survivin inhibitors have also undergone various developments and investigations. However, the therapeutic effectiveness of these inhibitors is still questionable. For example, the heat shock protein 90 (Hsp90) inhibitors such as 17-AAG and shepherdin have been developed and were claimed to down-regulate survivin as one of their most important therapeutic effects (81-83). Base on the fact that Hsp90 physically binds to survivin and prevents the degradation of survivin by the proteasome, it is expected that interference of the physical interaction between Hsp90 and survivin with Hsp90-specific inhibitor will promote survivin degradation through the proteasome (84). Surprisingly, our recent study reveals that targeting Hsp90 by pharmacological inhibitors, geldanamycin and 17-AAG, induces the expression of survivin in A549, HT-29 and HONE-1 cancer cells through both transcriptional and post-transcriptional mechanisms (85). Since Hsp90 interferes with multiple molecules such as Sp1, Sp3 (both transcriptional factors and positive regulators of survivin), p53 (negative regulator of survivin transcription) and 26S proteasome (negative regulator of survivin protein level) simultaneously (86, 87), down-regulation of survivin may not be a definite therapeutic effect of Hsp90 inhibitors as we previously thought. In fact, a recent study from Stingl et al. also demonstrates that two novel Hsp90 inhibitors, NVP-AUY922 and NVP-BEP800, induce the up-regulation of survivin in the human HT1080 fibrosarcoma cells in vitro (88).

The effectiveness of another indirect survivin inhibitor, terameprocol, has also been investigated in various pre-clinical and clinical studies (89, 90). At the molecular level, terameprocol competes with the transcriptional factor Sp1 for specific Sp1 DNA binding domains within gene promoter regions during DNA synthesis. By suppressing Sp1-regulated transcription of the survivin gene (and other genes such as VEGF and cdk1), terameprocol may induce tumor cell apoptosis (89). However, detailed effectiveness and safety profiles of the use of terameprocol in clinical situations are still under evaluation. Furthermore, it is hard to know whether the down-regulation of survivin plays the major role in inducing cancer cells death during terameprocol treatment. On the other hand, a tubulin-targeted compound, EM011, has shown the ability to down-regulate survivin expression and subsequently induce survivin-related cancer cell death. It has been demonstrated that EM011 suppresses the proliferation of various human lung cancer cells such as A549, H460 and H157 in vitro (91). At the cellular level, EM011 induces mitotic arrest at the G2/M phase by the activation of the spindle checkpoint. It is not surprising to know that EM011 is able to induce cell cycle arrest at the G2/M phase because most anti-mitotic compounds such as vincristine, colchicine and palcitaxel are capable of inducing similar cellular and molecular responses (92). However, it is interesting to note that this compound could down-regulate the expression of survivin in cancer cells, given that survivin expression was previously shown to be increased in cells treated with other anti-mitotic compounds in vitro (30). The underlying mechanism of this effect has not yet been determined and it is hard to know whether the down-regulation of survivin plays a major role in inducing cancer cells death during EM011 treatment. Importantly, whether the down-regulation of survivin is a non-specific therapeutic effect of EM011 or the result of reduced metabolic rate (reduced rate of protein synthesis) in cells during the pro-apoptotic stage would also require further investigation.

4. SUMMARY AND PERSPECTIVE

Survivin plays multiple roles in the promotion of cancer cell survival and the causation of cancer anti-drug responses. The advancement of survivin biology in the past decade did not, however, translate immediately to the successful development of clinically applicable survivin-inhibitors, as the availability of survivin inhibitors is still limited at this stage. Therefore, further development of the survivin-specific small molecule inhibitors is required and may be important for future cancer treatments. On the other hand, optimized survivin-targeting macromolecules may be used as an alternative way to treat cancers that express survivin. As we have mentioned that survivin plays different roles in different organelles in cancer cells, organelle transduction domain (PTD)-mediated survivin-specific macromolecular therapy may be useful in combination with different organelle-specific chemotherapeutic compounds in future clinical settings. More investment and investigation is needed to develop better survivin-targeted treatments.

5. ACKNOWLEDGEMENTS

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6. REFERENCES


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proliferation, and angiogenesis during human colorectal tumorigenesis. *Cancer*, 91(11), 2026-32 (2001)


46. M. Ikeguchi, J. Liu and N. Kaibara: Expression of survivin mRNA and protein in gastric cancer cell line
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**Abbreviations:** AE (adverse effect), IAP (inhibitor-of-apoptosis), INCENP (inner centromere protein), TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), YM155 (1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d]imidazolium bromide)

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