MINI-REVIEW

Survivin, a Promising Gene for Targeted Cancer Treatment

Fatemeh T Shamsabadi[1], Mohammad Reza Akbari Eidgahi[2]*, Parvaneh Mehrbod[3], Nasibeh Daneshvar[4], Zeenathul Nazariah Allaudin[5], Ahad Yamchi[6], Majid Shahbazi[7,8]*

Abstract

Drawbacks of conventional cancer treatments, with lack of specificity and cytotoxicity using current approaches, underlies the necessity for development of a novel approach, gene-directed cancer therapy. This has provided novel technological opportunities in vitro and in vivo. This review focuses on a member of an apoptosis inhibitor family, survivin, as a valuable target. Not only the gene but also its promoter are applicable in this context. This article is based on a literature survey, with especial attention to RNA interference as well as tumor-specific promoter action. The search engine and databases utilized were Science direct, PubMed, MEDLINE and Google. In addition to cell-cycle modulation, apoptosis inhibition, interaction in cell-signaling pathways, cancer-selective expression, survivin also may be considered as specific target through its promoter as a novel treatment for cancer. Our purpose in writing this article was to create awareness in researchers, emphasizing relation of survivin gene expression to potential cancer treatment. The principal result and major conclusion of this manuscript are that survivin structure, biological functions and applications of RNA interference systems as well as tumor-specific promoter activity are of major interest for cancer gene therapy.

Keywords: Survivin - gene therapy - RNA interference - tumor-specific promoter

Asian Pac J Cancer Prev, **17 (8)**, 3711-3719

Introduction

Cancer is one of the most life-threatening diseases worldwide. Cancer is a multiple genetic disease which occurs due to the up- and/or down-regulation of oncogenes and tumor-suppressor genes as well as deregulation of apoptosis pathways. In most cases, conventional anticancer therapies fail (or could not be effective due to the lack of specificity and cytotoxicity), therefore, novel improved therapies are urgently needed (Roche and Vahdat, 2010). On the other hand, cancer cells are illustrated as derailed cells due to a large variety of genetic mutations (Pecorino, 2012) resulting in an unbalance between cell survival and apoptosis.

As a consequence of cell cycle machinery deregulation, cancer cells proliferate in abnormal and uncontrolled format which results in tumor progression and invasiveness (Roberti et al., 2009). Today, considerable efforts have developed strategies to target apoptosis in cancer cells and other human diseases. Apoptosis or programmed cell death is an essential phenomenon in eliminating derailed cells, which occurs either in development and aging processes or as a defense mechanism (i.e., immune reactions) or when the cells are damaged by disease or noxious agents (Fan and Bergmann, 2008). The inactivation of apoptosis is fundamental to the cancer development.

The significance of genetic alterations in the control of cellular proliferation/apoptosis pathways in cancer development has been well established. For instance, over-expression of the anti-apoptotic protein (e.g. Bcl-2) due to chromosomal translocations results in e.g. breast cancer and B-cell lymphoma (Thomas et al., 2013). Presence of abnormal forms tumor suppressor p53 protein or its absence has also been linked to the development of various tumors (Stegh, 2012; Tokino et al., 2015). In addition, up-regulated expression of apoptosis inhibitors family prevents cancer cells in undergoing apoptosis (Fulda and Vucic, 2012). The inhibitor of apoptosis (IAP) proteins family is the most important regulators of apoptosis due to their function in both the intrinsic and extrinsic pathways, while their significant roles revealed in the survival of cancer cells and the progression of malignancies (Wong, 2011). IAP proteins exert a variety of biological activities that promote cancer cell survival and proliferation. The

*[1]Faculty of Medicine, Semnan University of Medical Sciences, [2]Semnan Biotechnology Research Center, Semnan University of Medical Sciences, Semnan, [3]Influenza research lab, Virology Department, Pasture Institute, Tehran, iran [4]Biological Sciences Building, University of Manitoba, Winnipeg, Manitoba R3T 2N2 Canada [5]Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia [6]Gorgan University of Agricultural Sciences and Natural Resources, Golestan, [7]Golestan University of Medical Sciences, [8]AryaTinaGene Bio Pharmaceutical Company, Gorgan, Golestan *For correspondence: shahbazimajid@yahoo.co.uk, mrakbari201177@yahoo.com*

expression of IAP protein family including XIAP, cIAP1, cIAP2 and survivin in human cancer cells has been investigated as a potential factor for chemo-resistance, based on their ability to inhibit the caspases as key molecules of the apoptotic machinery (Pop and Salvesen, 2009; Kaufmann et al., 2012). On the other hand, X chromosome-linked IAP is a direct caspase inhibitor, whereas cellular IAP proteins prevent the assembly of pro-apoptotic protein signaling complexes and the expression of anti-apoptotic molecules is mediated. Furthermore, mutations, amplifications and chromosomal translocations of IAP genes are coordinated with various malignancies (Fulda and Vucic, 2012; Kaufmann et al., 2012).

Recognition of the aberrant molecular processes underlying tumor formation has unraveled partially the generation of potential novel anticancer therapies. Survivin has well been characterized in both survival and apoptosis networks (Altieri, 2008). Survivin is indicated as a superior candidate in cancer gene therapy due to many reasons including high expression of survivin in tumor cells which has prognostic value (Duffy et al., 2007), its involvement in multiple cellular signaling pathways (i.e., promotes cell proliferation that causes cell death resistance) which contributes to the cancer growth and progression (Kanwar et al., 2011), promotes angiogenesis (Eberlein et al., 2013) and its responsibility for resistance to cytotoxic drugs (Wu et al., 2007; Faversani et al., 2014) and radiation (Yang et al., 2010b). Therefore, targeting survivin has been attracted as a possible point of therapeutic in halting cancer progression and cancer gene therapy.

Survivin Structure and Sequence

Survivin as a smallest member of the IAP family is composed of 142 amino acids and does not indicate significant sequence homology with known cellular proteins. The protein of survivin with homodimeric structure contains phosphorylation sites at Thr34 (cyclin-dependent kinase 1 (CDK1)); Thr117 (Aurora kinase B); Ser20 (protein kinase A (PKA)). The N-terminal end of survivin contains a bipartite dimer interface hydrophobic pocket, borealin and INCENP: which span residues Leu6-Trp10, Phe93-Leu102. In addition, survivin contains a putative nuclear export sequence (NES) at residues Val89–Leu98 (Figure 1), which results in driving the shuttling of survivin in and out of the nucleus. A single baculovirus IAP repeat (BIR) motif is a unique structural feature of survivin which was separated from the other members as well as α-helical coiled-coil portion extended in carboxyl terminus (Altieri, 2008). The protein sequence of survivin was evaluated by the online bioinformatics database and highlighted the NES as well as target kinases in green and yellow colors.

Survivin also harbors stretches of SMAC (Leu64, Leu87); X-linked IAP (XIAP) (Lys15–Met38); heat shock protein 90 (HSP90, Lys79–Lys90); Aurora kinase B (Asp70, Asp71); mitochondrial-targeting sequence (survivin-ΔEx-3 C terminus) that is required for binding of other interaction partners (Altieri, 2008).

As a structural characteristic of survivin like other members is the presence of baculovirus IAP repeat (BIR) domain that stretches from amino acid residues 15 to 87 at the N-terminus region. The domain has a conserved fragment as a ring finger motif at the carboxyl terminus containing cysteine/histidine residues which is incorporated with Zn2+ ion. The BIR motif is crucial for dimer formation and interacts with caspases (Ryan et al., 2009) which results in interference of apoptosis process, and thus promote tumor progression (Salvesen and Duckett, 2002).

The human survivin gene is located on chromosome 17q25 produce five different variants through the alternative splicing of its pre-mRNA which results in five distinct proteins including survivin (142 aa), survivin 2B (165 aa), survivin ΔEx3 (120 aa), survivin 3B (137 aa) and survivin 2α (74 aa) (Ryan et al., 2009). The X-ray crystal structure of the human survivin protein was provided by online protein data bank (PDB) which indicated a three-stranded anti-parallel β sheet that is surrounded by four α helices (Figure 2).

From the viewpoints of oncologist, survivin as a nodal protein is an attractive potential target in cancer treating due to its significant features such as over-expression in majority of cancer cells, a prognostic biomarker in cancer

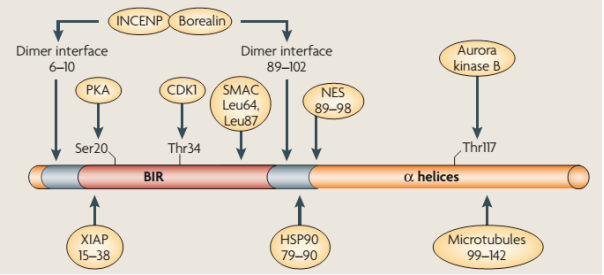

Figure 1. Primary Structure and Sequence of Survivin. The key domains and important sequences are indicated. The survivin protein sequence indicated in lower panel. Amino acids marked in blue represent the cysteine/histidine residues (Cx2Cx6Wx3Dx5Hx6C, where x is any amino acid) in BIR domain (Altieri, 2008). The nuclear export sequence (NES) is highlighted in green. The target kinases are highlighted in yellow. The residues are underlined represent the dimer interference fragments

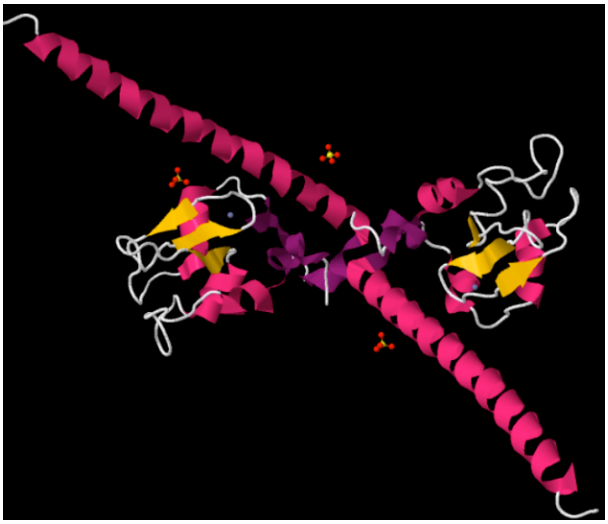

Figure 2. X-ray Crystal Structure of the Human Anti-Apoptotic Protein Survivin, a 16.5 kDa Protein

(Cai et al., 2014), as well as its involvement in multiple signaling pathways contributing to tumor maintenance.

Survivin Functions in Molecular Biology Networks

Survivin is known as multi-functional protein which participates in at least three homeostatic networks including mitosis regulation, apoptosis inhibition (Mita et al., 2008; Altieri, 2013) and cellular stress response (Altieri et al., 2015). The survivin obligation was briefly demonstrated in the following context.

Survivin as cell-cycle modulator:

The function of survivin in regulating cell division is attributed to the chromosomal passenger complex which interacts with other proteins such as Aurora B kinase, INCENP and Borealin (van der Horst and Lens, 2014). Indeed, in this complex, survivin supports proliferation through spindle stability (Altieri, 2008) and accurate sister chromatid segregation. In addition, it promotes mitosis during the spindle assembly checkpoint by controlling chromatin-associated spindle formation and kinetochore-microtubule attachment (Raab et al., 2015). It is interesting to note that transcription of survivin gene is strictly regulated by a cell cycle-dependent manner and cell cycle homology inside the promoter region, which leads to greatest expression in the G2/M phase of the cell cycle. Likewise, survivin anti-apoptotic action is due to loss of control over defects in the structure of mitotic spindle or in chromosome configuration during cell division (Li et al., 1998).

Survivin as inhibitor of apoptosis:

Deregulation of apoptosis is known as a major function of survivin which is demonstrated in several in vitro and in vivo experiments. Survivin counteracts with both intrinsic (e.g., Bcl-2, Bcl-xl, Mcl-1, BAX, caspase 9) and extrinsic (e.g., FAS, TRAIL, caspase-8, FLIP, caspase 3) apoptosis pathways and other mediators including p53, methotrexate and IL-3 (Ryan et al., 2009; Liu et al., 2011).

Survivin is unable to directly attach to caspases due to the lack of linker sequence in upstream of the BIR domain and the ''hook and sinker'' domain which mediates the binding of IAPs family to caspases except XIAP. Hence, the survivin-XIAP complex enhances XIAP stability, activates NFκB signaling pathway which results in the inhibition of caspase-3 and -9, suppresses apoptosis and accelerates tumor progression, in vivo. A further cytoprotective mechanism of survivin is associated with SMAC mitochondrial pro-apoptotic protein. As a result of the binding of survivin to SMAC, the SMAC antagonism for XIAP will reduced. The free IAP, therefore, can interact with caspases and inhibit apoptosis (Fulda and Vucic, 2012).

Survivin in cellular stress response:

The responsibility of survivin in the cellular stress is well demonstrated. Survivin involves with a variety of chaperone molecules such as Hsp90 (Altieri et al., 2015) and Hsp60 (Ghosh et al., 2008; Kelly et al., 2011) which provides adaptation under cellular stress conditions by maintaining survivin stability, folding and subcellular trafficking, in vivo.

Interestingly, there is a correlation between survivin and the Fas ligand (FasL) expression in cancer cells through up-regulation of specific protein-1-mediated gene transcription. On the other hand, survivin enhances the DNA binding of the transcription factor SP1 to the FASL promoter and up-regulated SP1 through phosphorylation at Ser and Thr residues. Moreover, survivin facilitates suppression of immune system in cancer cells by inhibiting FAS-mediated apoptosis, and also attack immune cells via FASL induction (Asanuma et al., 2004).

Notably, survivin protein possess the nuclear-cytoplasmic shuttling function which is associated with nuclear localization signal. Indeed, survivin exported from the nucleus by directly interaction with the nuclear export receptor CRM1 and Ran-guanosine 5'-triphosphate (RanGTP) (Chan et al., 2010). As a result of this function, survivin eliminates its cytoprotective effect toward the apoptotic mediators and thus commits a cell to apoptosis. The cytoplasmic localization of survivin is essential for its apoptosis inhibition activity, while survivin interaction with CRM1 is essential for centromer localization (Knauer et al., 2007). Moreover, variants of survivin localization are differentially regulated; for instance, survivin-2B as a shuttling protein is mainly localized in the cytoplasm, whereas, survivin-ΔEx3 by a bipartite nuclear localization signals shows strong nuclear localization (Chan et al., 2010).

The most recent review was indicated the association of survivin molecule in different cell-signaling mechanisms such as therapeutic modulation of survivin which is critically regulated by interaction with prominent cell-signaling pathways [HIF-1α, HSP90, PI3K/AKT, mTOR, ERK, tumor suppressor genes (p53, PTEN), oncogenes (Bcl-2, Ras)] and a wide range of growth factors (EGFR, VEGF, among others) (Kanwar et al., 2011). The current review demonstrated the main survivin interactions with molecules which are contributed to cell-cycle and apoptosis processes, based on the STRING tool of Expasy website (Table 1).

What we know about survivin is largely based upon empirical studies that investigate how its precise function in mitotic regulation through AURKB, CDCA8, INCENP, CDK1, AURKA, FOXM1, SGOL2 molecules. Moreover, CDK4 and XIAP molecules are found to be influencing apoptosis inhibitor activity of survivin which have been explored in several studies. On the other hand, according to this table, the importance of survivin gene and its association in regulation as well as controlling cell proliferation was demonstrated, which from the other point of view, each of these molecules could be considered in cancer gene therapy.

Application of Survivin in Gene Therapy

Due to the drawbacks of cancer conventional therapies, i.e., cytotoxicity, lack of selectivity and resistance to chemotherapeutic drugs, an efficient tool for treating cancer is required. Based on the impacts of genes in cancer biology, gene therapy approach is developed. Compared

Table 1. Survivin-Interacting Molecules

Molecules	Symbol	Biological effects	Ref.
Aurora kinase B (344 aa)	AURKB	Survivin promotes mitosis through direct interaction with AURKB as a mitotic regulator and enhances its activity which contributes to the aberrant growth of cancer cells. The chromosome passenger complex containing survivin and AURKB regulate chromosome segregation from the centromeres.	(Baratchi et al., 2010)
Cell division cycle associated 8 (280 aa)	CDCA8	As a component of a chromosomal passenger complex interacts with INCENP, Survivin and Aurora B kinase which is required for the chromatin-induced microtubule stabilization and spindle formation.	(Altieri, 2008)
Inner centromer protein (918 aa)	INCENP	Survivin has a key regulatory role in the organization of chromosomal passenger complex through its interacting subunits INCENP and Borealin/Dasra B that leads to the spindle apparatus formation during anaphase. Indeed, it facilitates the localization of the Aurora B kinase-INCENP complex to the inner chromosomal region of centromeres at the early stages of mitosis.	(van der Horst and Lens, 2014)
Cyclin-dependent kinase 1 (297 aa)	CDK1	Cdk1 stabilizes survivin by phosphorylation on Thr34 from proteasomal degradation by Cdc25B in pro-metaphase and metaphase.	(Unruhe et al., 2015)
Cyclin-dependent kinase 4 (303 aa)	CDK4	Survivin initiates pro-caspase 3/p21 complex formation as a result of interaction with Cdk4 to resist Fas-mediated cell death.	(Tarasewicz et al., 2014)
Polo-like kinase 1 (603 aa)	PLK1	PLK1 phosphorylation is required for progression through the G2/M transition. It is a critical for centrosome duplication, and both PLK1 and AURKB are involved in bipolar microtubule spindle attachment to the centromeric kinetochores. PLK1 depletion is associated with decreased survivin protein expression and increased level of active caspase-3 in hepatocellular carcinoma tissue.	(Raab et al., 2015)
Aurora kinase A (403 aa)	AURKA	As a mitotic serine/threonine kinase contributes to the regulation of cell cycle during anaphase and/or telophase.	(Fu et al., 2007)
Cell division cycle 20 homolog (499 aa)	CDC20	It is essential cell division regulator which initiates chromatid separation through anaphase promoting complex activation. CDC20 inhibition repressed several genes that are critical to tumor growth and survival including survivin, CDC25C and c-Myc.	(Xie et al., 2015)
Forkhead box M1 (801 aa)	FOXM1	FoxM1 acts as a transcriptional activator factor which is essential for transcription of the mitotic regulatory genes such as Cdc25B, Aurora B kinase, survivin, PLK1, centromere protein A (CENPA), and CENPB.	(Wang et al., 2005)
X-linked inhibitor of apoptosis (497 aa)	XIAP	Anti-apoptotic function of survivin relies on the interaction with XIAP. The E3 ubiquitin ligase activity of XIAP increases XIAP stability, activates NFκB pathway, synergistically inhibits caspase-3 and -9, suppresses apoptosis, and accelerates tumor progression in vivo.	(Moraes et al., 2013)
Shugoshin-like 2 (1265 aa)	SGOL2	It regulates the stability of survivin-INCENP interaction. Lack of Sgo2 has a more penetrant effect on the localization of survivin than on the two other Passenger proteins INCENP and Aurora B. Sgo2 colocalizes with the passenger proteins in early mitosis and promotes their efficient recruitment onto centromeres and telomeres.	(Vanoosthuyse et al., 2007)

to the conventional methods, gene therapy helps to treat the cause of the disease.

Existing research recognizes the critical challenge in targeting of survivin for therapeutic purposes owing to not a cell surface protein, does not have an intrinsic enzymatic activity as well as few potential drugable sites on survivin protein. Therefore, therapeutic strategies were developed to target survivin expression and/or function based on the inhibiting of survivin promoter such as YM155, inhibition of protein translation and interfering with survivin function. In general, these approaches are divided into transcriptional (e.g., antisense oligonucleotides, ribozymes and siRNA) and post-translational (e.g., dominant-negative mutants, small molecule antagonists and immunotherapy) stages (Ryan et al., 2009; Mobahat et al., 2014). More details for each mechanisms were described and reviewed in (Soleimanpour and Babaei, 2014).

Survivin and RNA interference

Table 2. Preclinical RNA Interference Studies on the Therapeutic Potential Of Survivin

Tumor	Preclinical models	Ref.
Thymic lymphoma	*In vitro/in vivo*	(Kanwar et al., 2001)
Gastric	*In vitro/in vivo*	(Tu et al., 2003; Li et al., 2015b)
Bladder	*In vitro*	(Ning et al., 2004; Yang et al., 2010b; Kunze et al., 2013)
Sarcoma	*In vitro*	(Kappler et al., 2004; Kappler et al., 2005)
HeLa	*In vitro/in vivo*	(Trabulo et al., 2011; Li et al., 2015a)
Breast	*In vitro/in vivo*	(Uchida et al., 2004; Croci et al., 2008; Yang et al., 2011)
Prostate	*In vitro/in vivo*	(Shen et al., 2009; Liu et al., 2010; Cavalieri et al., 2015)
Lung	*In vitro/in vivo*	(Lu et al., 2004; Liu et al., 2009; Yang et al., 2010a; Chen et al., 2012)
Hepatocellular	*In vitro/in vivo*	(Cheng et al., 2005; He et al., 2007; Shen et al., 2014)

Survivin is well distributed by inhibiting apoptosis and enhancing cell viability functions. Likewise, its selective-overexpression is demonstrated in the majority of cancer cells which correlates with tumor grade, recurrence risk and survival. The knockdown of genes that contribute to tumor development and progression shows great potential as an anticancer strategy (Ramachandran and Ignacimuthu, 2012; Felipe et al., 2013).

Gene-directed cancer therapy by RNA interference (RNAi) is a major area of interest in the field of cancer gene therapy as widely provided novel technological opportunities in vitro and in vivo. Moreover, gene targeting by means of this technique is introduced as a strategy to explore gene function because of its prominent efficacy and specificity (Uchida et al., 2004; Karami et al., 2014). Based on the encouraging findings, most survivin preclinical investigations according to RNA interference are summarized in Table 2.

According to these researches, knockdown of survivin using multiple approaches of RNAi technique displayed remarkably reduced cell proliferation as a result of susceptibility to apoptosis in different cancer cell lines (Cheng et al., 2005; Croci et al., 2008; Shen et al., 2009; Yang et al., 2010b) as well as lower tumor formation in vivo experiments (Kanwar et al., 2001; Uchida et al., 2004; Liu et al., 2009; Shen et al., 2009).

The association between survivin expression and radiosensitivity has been described in lung (Lu et al., 2004), sarcoma (Kappler et al., 2005) and non-small cell lung (Yang et al., 2010a) cancer cell lines. It was reported that irradiation of human umbilical vein endothelial cells suppressed the survivin promoter which resulted in the reduction of survivin expression in independently cell-cycle manner (Lu et al., 2004). In addition, they demonstrated that p53 over-expression is associated with decrease of survivin expression in the same cell line, whiles siRNA-mediated down-regulation of survivin caused to cell viability reduction in irradiated H460 lung cancer cells. By drawing on the concept of p53 association in radiosensitization, Kappler and co-workers have been able to demonstrate that survivin knockdown induced radiosensitivity in wt-p53 cell line (A-204), but not in a mt-p53 sarcoma cell line (Kappler et al., 2005). These findings indicated the impact and association of p53 pathway on the mechanism of radiosensitization induced by targeting survivin.

The cell-cycle modulator function of survivin is well distributed. Following the down-regulation of survivin expression, cell-cycle analysis showed contradictory results. Some studies have shown that suppressing survivin resulted in a specific G2/M arrest in bladder and Hela cancer cells (Ning et al., 2004; Li et al., 2015a), while a large proportion of lung cancer cells were blocked in the G1 phase, leading to the decrease of cells in the S phase (Chen et al., 2012). This finding do not support the previous research, but they are broadly consistent with the effect of survivin down-regulation on the inhibition of cell proliferation through the increase of apoptosis as well as decrease in the mitotic activity of the cells .

Subsequent silencing of survivin leading to blockage of the apoptosis inhibitory activity of survivin, which is attributed to the activation of caspase-3, -7 and -9 (Kappler et al., 2005; Yang et al., 2010b; Chen et al., 2012). On the other hand, survivin suppression in cancer cells increases the susceptibility of the cells to apoptotic factors.

Obviously, resistance to chemotherapy is associated with reduced susceptibility to apoptosis. Evidence shows a relationship between cytotoxic drugs as chemotherapy agents and expression of survivin in most malignancies. Therefore, the combination of target-specific molecular-based treatments with chemotherapy or radiotherapy could increase the efficacy of the conventional therapies and reverse acquired treatment resistances (Kunze et al., 2013). On the other hand, cancer cells could be sensitized to chemotherapy through siRNA and/or shRNA-mediated knockdown of target genes. For instance, simultaneous suppression of survivin with chemotherapy drugs such as adrymycin, sorafenib, mitomycin C and cisplatin (platinol) were demonstrated the synergistic effects in inhibiting cancer cell proliferation as well as enhancing the sensitization of breast (Yang et al., 2011), hepatocellular (Shen et al., 2014), bladder (Yang et al., 2010b; Kunze et al., 2013) and prostate (Shen et al., 2009) cancer cells, respectively. In fact, these results imply that co-administration of survivin RNA interference strategies with chemo- or radio-therapy could be efficient and valuable as a potent multi-targeted gene therapy for different type of cancers.

At the moment, abundant gene therapy trials are in progress, majority of them (approximately 70%) being in the field of cancer. Today, several gene therapy approaches

Table 3. Overview of Applied Survivin Promoter in Cancer Gene Therapy Research

Expression Promoter length	Target gene	Cancer cell	Ref.
269-nt	Reverse caspase 3	Breast, pancreatic	(Yang et al., 2004)
260-nt	E1	Glioma	(Van Houdt et al., 2006)
260-nt	E1, E4	Biliary tract cholangio	(Zhu et al., 2006a)
980-nt	PUMA	Breast	(Wang et al., 2009)
260-nt	Luciferase reporter gene	Ovarian, breast, pancreatic, melanoma	(Zhu et al., 2004)
270-nt	VEGF receptor, sFlt-1	Kidney	(Namgung et al., 2010)
Silencing			
983-nt	Stathmin	Cervical, osteosarcoma	(Zhang et al., 2006)
980-nt	hTERT	Cervical	(Wang et al., 2007)
980-nt	UHRF1	Breast	(Fang et al., 2012)
980-nt	Notch3	Leukemia	(Xiang et al., 2012)

targeting survivin were indicated which some of them passed proof-of-principle in preclinical studies. Among the mentioned approaches, antisense oligonucleotide (e.g., LY2181308) by interfering transcriptional process was applied in phase II clinical trial of leukemia and prostate cancers. Similarly, trials of small molecule with inhibition function such as YM155 and EM-1421 (Terameprocol) are going in patients with lymphoma, prostate and leukemia, respectively. Besides, administration of survivin based on immunotherapies have been started phase I and II clinical trials in melanoma, colon, breast and cervical cancers (Ryan et al., 2009).

Since the preferential expression of survivin in a variety of cancer cells but not in normal tissues, these findings may lead to new directions for the targeting the survivin pathway alone through its suppression by different RNAi approaches (e.g., shRNA and siRNA), or with other cytotoxic drugs may have an impact on future gene therapies.

Survivin Promoter

Over the years, cancer biology researches recognized various genes that are typically silent in normal tissues but are highly up-regulated in various cancers. Exploiting these tumor-specific abnormalities might represent a powerful way to improve the targeting achieved by tumor-directed vectors. On the other hand, application of tumor specific/or selective promoter for enhancing the specificity of treatment is another advantages of gene therapy approach. These days, RNA interference (RNAi) as a post-transcriptional gene therapy approach is emphasized in cancer gene therapy. Amongst RNAi methods, DNA vector-based short hairpin RNA technique is recently highlighted for several features especially its more extended gene knockdown effect, as well as definite silencing via specific promoter (Wang et al., 2013).

Over-expression of survivin was demonstrated in various human cancers (Church and Talbot, 2012) such as breast (Jha et al., 2012), small-cell lung (Chen et al., 2014b), esophagus (Xia et al., 2015), colon (Hernandez et al., 2011), gastric (Chen et al., 2014a) as well as its association with poor patient survival (Jha et al., 2012; Park et al., 2012). Since, the expression of survivin is transcriptionally activated; consequently, its promoter might be a cancer-specific promoter with utility in gene therapy or oncolytic viral replication (Bao et al., 2002).

The human survivin promoter has been investigated in extensive detail. Analysis of the 5' flanking region of the survivin gene revealed the presence of a TATA-independent promoter containing a CpG island of approximately 250 nucleotides, three cell cycle dependent elements (CDE) (GGCGG at - 6,-12,-171), one cell cycle homology region (CHR) (ATTTGAA at -42) and numerous Spl sites (Li and Altieri, 1999). In addition, two transcription sites at positions -72 and between -57 and -61 from the start codon were established by primer extension and SI nuclease mapping experiments. The truncated examination of minimal promoter region revealed the presence of enhancer as well as repressor sequences along the proximal 230 nucleotide of the survivin gene (Li and Altieri, 1999; Zhu et al., 2004). Notably, the basal transcriptional activity of the promoter is associated with the Spl sequences at positions -171 and -151, as mutations of these sites abolished the activity by 63-80% (Chen et al., 2011). There is a high degree of conservation of proximal promoter regions, including the CpG Island, between the mouse and the human promoters.

In the earliest experiment in survivin promoter targeting, a 1092 nucleotide fragment of the human survivin promoter was applied to express the secreted alkaline phosphatase (SEAP) gene in ovarian cancer. The survivin promoter was capable to target gene expression specifically to cancer cells sparing the normal tissues in in vivo (Bao et al., 2002). The significance of survivin promoter for transcriptional targeting of cancer gene therapy was subsequently verified by employing a 260bp survivin promoter targeted in adenovirus vector which demonstrated the desirable "tumor on" and "liver off" profile (Zhu et al., 2004). Later, the strong cancer specific activity, 200-fold more than the CMV promoter, was proved by a 977bp promoter fragment in animal model of lung cancer (Chen et al., 2004). The survivin promoter was further engrossed due to the putative binding sites for transcription factor such as hypoxia-inducible factor-1α (HIF-1α; GTGC at -136 and GTGCGC at -21), E2F (GCGC at -151 and +4) as well as p53 (CATG at -9) (Chen et al., 2009), which is another reason for considering the promoter in cancer gene therapy applications.

Till date several researches verified the ability of the human survivin promoter to specifically target different type of cancers from tumor cells to small animal models (Zhu et al., 2005; Li et al., 2006; Van Houdt et al., 2006). In the recent past, survivin promoter regulated oncolytic

viruses have shown tremendous potential as therapeutic agents for different forms of carcinomas (Li et al., 2006; Zhu et al., 2006a; Zhu et al., 2006b; Ulasov et al., 2007). Moreover, the promoter was also highly effective in shRNA based gene therapy approaches. For instance, a survivin promoter-driven siRNA expression vector silenced the human Telomerase Reverse Transcriptase (TERT) gene in cervical carcinomas followed by enhancing radio-sensitivity in vitro (Wang et al., 2007). In another experience, assessment of both hTERT and survivin promoters showed a comparable expression in cancer cells but a higher degree of specificity.

High cancer specific activity of survivin promoter was demonstrated in vitro and in vivo (Bao et al., 2002; Chen et al., 2004; Altieri, 2013). Moreover, the promoter was applied for expressing as well as silencing of target genes through vector-based shRNA. An overview of these researches was presented in Table 3.

The promoter of the human survivin gene has emerged as a favorable promoter of choice for gene therapy applications. In the latest laboratory experiment, our research group already designed a bi-directional survivin-tumor specific promoter which could be applied for the concurrent silencing, over-expression as well as up/down-regulate strategies that target two genes at the same time.

Conclusions

The last two decades have seen a growing trend towards gene therapy in several diseases. Among the novel drugs, survivin is broadly explored in cancer gene therapy due to the importance of survivin in tumorigenesis, tumor progression and poor prognosis, as well as tumor resistance to various apoptotic stimuli. This review described survivin structure and its interaction by other cellular proteins as well as a growing panel of preclinical studies that target survivin as a novel anti-cancer gene therapy. The tumor-selective promoter activity of survivin was also depicted and explained. In fact, survivin is known as a potential candidate in cancer gene therapy due to its application as tumor biomarker, combination therapy by its inhibition targeting and tumor-selective activity of its promoter.

The proteins which induce apoptosis inhibition in tumor cells will sense specific pathways for tumor formation which can redirect them into cell survival. Disclosing the synergy between transformation and the factors inducing tumor-selective apoptosis will provide a better understanding of the processes underlying cancer in developing novel anticancer agents. The clinical trials, phases I and II, have provided the hope that the current research field will become critical in the development of novel improved anticancer strategies.

References

Altieri DC (2008). Survivin, cancer networks and pathway-directed drug discovery. *Nat Rev Cancer*, **8**, 61-70.

Altieri DC (2013). Targeting survivin in cancer. *Cancer lett*, **332**, 225-8.

Asanuma K, Tsuji N, Endoh T, et al (2004). Survivin enhances Fas ligand expression via up-regulation of specificity protein 1-mediated gene transcription in colon cancer cells. *J Immunol*, **172**, 3922-9.

Bao R, Connolly DC, Murphy M, et al (2002). Activation of cancer-specific gene expression by the survivin promoter. *J Natl Cancer I*, **94**, 522-8.

Baratchi S, Kanwar RK, Kanwar JR (2010). Survivin: a target from brain cancer to neurodegenerative disease. *Crit Rev Biochem Mol*, **45**, 535-54.

Cai J, Fu S, Tu Z, et al (2014). Survivin Gene Functions and Relationships between Expression and Prognosis in Patients with Nasopharyngeal Carcinoma. *Asian Pac J Cancer Prev*, **16**, 2341-5.

Cavalieri F, Beretta GL, Cui J, et al (2015). Redox-sensitive PEG–polypeptide nanoporous particles for survivin silencing in prostate cancer cells. *Biomacromolecules*, **16**, 2168-78.

Chan KS, Wong CH, Huang YF, et al (2010). Survivin withdrawal by nuclear export failure as a physiological switch to commit cells to apoptosis. *Cell Death Differ*, **1**, 57.

Chen J-S, Liu JC, Shen L, et al (2004). Cancer-specific activation of the survivin promoter and its potential use in gene therapy. *Cancer Gene Ther*, **11**, 740-7.

Chen J, Li T, Liu Q, et al (2014a). Clinical and prognostic significance of HIF-1alpha, PTEN, CD44v6, and survivin for gastric cancer: a meta-analysis. *PloS one*, **9**, 91842.

Chen P, Zhu J, Liu D-y, et al (2014b). Over-expression of survivin and VEGF in small-cell lung cancer may predict the poorer prognosis. Med Oncol, **31**, 1-7.

Chen XQ, Yang S, Li ZY, et al (2012). Effects and mechanism of downregulation of survivin expression by RNA interference on proliferation and apoptosis of lung cancer cells. *Mol Med Rep*, **5**, 917-22.

Chen YQ, Zhao CL, Li W (2009). Effect of hypoxia-inducible factor-1α on transcription of survivin in non-small cell lung cancer. *J Exp Clin Canc Res*, **28**, 29.

Chen Y, Wang X, Li W, et al (2011). Sp1 upregulates survivin expression in adenocarcinoma of lung cell line A549. *Anat Rec*, **294**, 774-80.

Cheng SQ, Wang WL, Yan W, et al (2005). Knockdown of survivin gene expression by RNAi induces apoptosis in human hepatocellular carcinoma cell line SMMC-7721. *World J Gastroentero*, **11**, 756-9.

Church DN, Talbot DC (2012). Survivin in solid tumors: rationale for development of inhibitors. *Curr Oncol Rep*, **14**, 120-8.

Croci DO, Cogno IS, Vittar NBR, et al (2008). Silencing survivin gene expression promotes apoptosis of human breast cancer cells through a caspase-independent pathway. *J Cell Biochem*, **105**, 381-90.

Duffy MJ, O D, Norma, Brennan DJ, et al (2007). Survivin: a promising tumor biomarker. *Cancer Lett*, **249**, 49-60.

Eberlein C, Opoku-Ansah G, Legg J, et al (2013). Survivin regulates endothelial cell proliferation, survival and angiogenesis. *Cancer Res*, **73**, 1616.

Fan Y, Bergmann A (2008). Apoptosis-induced compensatory proliferation. The Cell is dead. Long live the Cell. *Trends Cell Biol*, **18**, 467-73.

Fang L, Shanqu L, Ping G, et al (2012). Gene therapy with RNAi targeting UHRF1 driven by tumor-specific promoter inhibits tumor growth and enhances the sensitivity of chemotherapeutic drug in breast cancer *in vitro* and *in vivo*. *Cancer Chemoth Pharm*, **69**, 1079-87.

Faversani A, Vaira V, Moro GP, et al (2014). Survivin family proteins as novel molecular determinants of doxorubicin resistance in organotypic human breast tumors. *Breast Cancer Res*, **16**, 55.

Felipe AV, Oliveira J, Chang P, et al (2013). RNA interference:

a promising therapy for gastric cancer. *Asian Pac J Cancer Prev*, **15**, 5509-15.
Fu J, Bian M, Jiang Q, et al (2007). Roles of Aurora kinases in mitosis and tumorigenesis. *Mol Cancer Res*, **5**, 1-10.
Fulda S, Vucic D (2012). Targeting IAP proteins for therapeutic intervention in cancer. *Nature Rev Drug Discov*, **11**, 109-24.
Ghosh JC, Dohi T, Kang BH, et al (2008). Hsp60 regulation of tumor cell apoptosis. *J Biol Chem*, **283**, 5188-94.
He S-Q, Rehman H, Gong M-g, et al (2007). Inhibiting survivin expression enhances TRAIL-induced tumoricidal activity in human hepatocellular carcinoma via cell cycle arrest. *Cancer Biol Ther*, **6**, 1258-68.
Hernandez JM, Farma JM, Coppola D, et al (2011). Expression of the antiapoptotic protein survivin in colon cancer. *Clin Colorectal Cancer*, **10**, 188-93.
Jha K, Shukla M, Pandey M (2012). Survivin expression and targeting in breast cancer. *Surg Oncol*, **21**, 125-31.
Kanwar JR, Kamalapuram SK, Kanwar RK (2011). Targeting survivin in cancer: the cell-signalling perspective. *Drug Discov Today*, **16**, 485-94.
Kanwar JR, Shen W-P, Kanwar RK, et al (2001). Effects of survivin antagonists on growth of established tumors and B7-1 immunogene therapy. *J Natl Cancer I*, **93**, 1541-52.
Kappler M, Bache M, Bartel F, et al (2004). Knockdown of survivin expression by small interfering RNA reduces the clonogenic survival of human sarcoma cell lines independently of p53. *Cancer Gene Ther*, **11**, 186-93.
Kappler M, Taubert H, Bartel F, et al (2005). Radiosensitization, after a combined treatment of survivin siRNA and irradiation, is correlated with the activation of caspases 3 and 7 in a wt-p53 sarcoma cell line, but not in a mt-p53 sarcoma cell line. *Oncol Rep*, **13**, 167-72.
Karami H, Baradaran B, Esfehani A, et al (2014). Down-regulation of Mcl-1 by small interference RNA induces apoptosis and sensitizes HL-60 leukemia cells to etoposide. *Asian Pac J Cancer Prev*, **15**, 629-35.
Kaufmann T, Strasser A, Jost P (2012). Fas death receptor signalling: roles of Bid and XIAP. *Cell Death Differ*, **19**, 42-50.
Kelly RJ, Lopez-Chavez A, Citrin D, et al (2011). Impacting tumor cell-fate by targeting the inhibitor of apoptosis protein survivin. *Mol Cancer*, **10**, 1186.
Knauer SK, Kramer OH, Knosel T, et al (2007). Nuclear export is essential for the tumor-promoting activity of survivin. *FASEB J*, **21**, 207-16.
Kunze D, Erdmann K, Froehner M, et al (2013). Enhanced inhibition of bladder cancer cell growth by simultaneous knockdown of antiapoptotic Bcl-xL and survivin in combination with chemotherapy. *Int J Mol Sci*, **14**, 12297-312.
Li B, Liu X, Fan J, et al (2006). A survivin-mediated oncolytic adenovirus induces non-apoptotic cell death in lung cancer cells and shows antitumoral potential *in vivo*. *J Gene Med*, **8**, 1232-42.
Li F, Altieri D (1999). Transcriptional analysis of human survivin gene expression. *Biochem J*, **344**, 305-11.
Li F, Ambrosini G, Chu EY, et al (1998). Control of apoptosis and mitotic spindle checkpoint by survivin. *Nature*, **396**, 580-4.
Li Y, Liu D, Zhou Y, et al (2015a). Silencing of Survivin Expression Leads to Reduced Proliferation and Cell Cycle Arrest in Cancer Cells. *J Cancer*, **6**, 1187.
Li Y, Zhou Y, Zheng J, et al (2015b). Downregulation of survivin inhibits proliferation and migration of human gastric carcinoma cells. *Int J Clin Exp Pathol*, **8**, 1731.
Liu G-F, Zhao Q-G, Si L, et al (2009). Effects of survivin interference RNA on non-small cell lung carcinoma. *Clin Invest Med*, **32**, 225-31.
Liu X, Gao R, Dong Y, et al (2010). Survivin gene silencing sensitizes prostate cancer cells to selenium growth inhibition. *BMC Cancer*, **10**, 418.
Liu Z, Cheng M, Cao MZ (2011). Potential targets for molecular imaging of apoptosis resistance in hepatocellular carcinoma. *Biomed Imag Interv J*, **7**, 5.
Lu B, Mu Y, Cao C, et al (2004). Survivin as a therapeutic target for radiation sensitization in lung cancer. *Cancer Res*, **64**, 2840-5.
Mita AC, Mita MM, Nawrocki ST, et al (2008). Survivin: key regulator of mitosis and apoptosis and novel target for cancer therapeutics. *Clin Cancer Res*, **14**, 5000-5.
Mobahat M, Narendran A, Riabowol K (2014). Survivin as a preferential target for cancer therapy. *Int J Mol Sci*, **15**, 2494-516.
Moraes GN, Vasconcelos FC, Delbue D, et al (2013). Analysis of survivin, XIAP and FoxM1 as potential chemoresistance factors in breast cancer cells. *BMC Proc*, **7**, 27.
Namgung R, Brumbach JH, Jeong JH, et al (2010). Dual bio-responsive gene delivery via reducible poly (amido amine) and survivin-inducible plasmid DNA. *Biotechnol Lett*, **32**, 755-64.
Ning S, Fuessel S, Kotzsch M, et al (2004). siRNA-mediated down-regulation of survivin inhibits bladder cancer cell growth. *Int J Oncol*, **25**, 1065-136.
Park DS, Hwang KE, Shim H, et al (2012). Elevated survivin is associated with a poor response to chemotherapy and reduced survival in lung cancer with malignant pleural effusions. Clin Exp Metastas, 29, 83-9.
Pecorino L 2012. Molecular biology of cancer: mechanisms, targets, and therapeutics, Oxford University Press.
Pop C, Salvesen GS (2009). Human caspases: activation, specificity, and regulation. *J Biol Chem*, **284**, 21777-81.
Raab M, Krämer A, Hehlgans S, et al (2015). Mitotic arrest and slippage induced by pharmacological inhibition of Polo-like kinase 1. *Mol Oncol*, **9**, 140-54.
Ramachandran PV, Ignacimuthu S (2012). RNA interference as a plausible anticancer therapeutic tool. *Asian Pac J Cancer Prev*, **13**, 2445-52.
Roberti A, Macaluso M, Giordano A (2009). Alterations in cell cycle regulatory genes in breast cancer. In 'Breast Cancer in the Post-Genomic Era', Eds Springer, 55-77
Roche H, Vahdat L (2010). Treatment of metastatic breast cancer: second line and beyond. *Ann Oncol*, **22**, 1000-1010.
Ryan BM, O'Donovan N, Duffy MJ (2009). Survivin: a new target for anti-cancer therapy. *Cancer Treat Rev*, **35**, 553-62.
Salvesen GS, Duckett CS (2002). IAP proteins: blocking the road to death's door. *Nature Rev Mol Cell Biol*, **3**, 401-10.
Shen J, Liu J, Long Y, et al (2009). Knockdown of survivin expression by siRNAs enhances chemosensitivity of prostate cancer cells and attenuates its tumorigenicity. *Acta Bioch Bioph Sin*, **41**, 223-30.
Shen J, Sun H, Meng Q, et al (2014). Simultaneous inhibition of tumor growth and angiogenesis for resistant hepatocellular carcinoma by co-delivery of sorafenib and survivin small hairpin RNA. *Mol Pharm*, **11**, 3342-51.
Soleimanpour E, Babaei E (2014). Survivin as a potential target for cancer therapy. *Asian Pac J Cancer Prev*, **16**, 6187-91.
Stegh AH (2012). Targeting the p53 signaling pathway in cancer therapy-the promises, challenges and perils. *Expert Opin Ther targets*, **16**, 67-83.
Tarasewicz E, Hamdan R, Straehla J, et al (2014). CDK4 inhibition and doxorubicin mediate breast cancer cell apoptosis through Smad3 and survivin. *Cancer Biol Ther*, **15**, 1301-11.
Thomas S, Quinn BA, Das SK, et al (2013). Targeting the Bcl-2 family for cancer therapy. *Expert Opin Ther targets*, **17**,

61-75.
Tokino T, Idogawa M, Sasaki Y (2015). P53 pathway and cancer: From bench to clinic. *Pers Med Universe*, **4**, 1-3.
Trabulo S, Cardoso A, Santos-Ferreira T, et al (2011). Survivin silencing as a promising strategy to enhance the sensitivity of cancer cells to chemotherapeutic agents. *Mol Pharm*, **8**, 1120-31.
Tu SP, Jiang XH, Lin MC, et al (2003). Suppression of survivin expression inhibits in vivo tumorigenicity and angiogenesis in gastric cancer. *Cancer Res*, **63**, 7724-32.
Uchida H, Tanaka T, Sasaki K, et al (2004). Adenovirus-mediated transfer of siRNA against survivin induced apoptosis and attenuated tumor cell growth *in vitro* and *in vivo*. *Mol Ther*, **10**, 162-71.
Ulasov IV, Zhu ZB, Tyler MA, et al (2007). Survivin-driven and fiber-modified oncolytic adenovirus exhibits potent antitumor activity in established intracranial glioma. *Hum Gene Ther*, **18**, 589-602.
Unruhe B, Schröder E, Wünsch D, et al (2015). An old flame never dies: survivin in cancer and cellular senescence. Gerontology.
van der Horst A, Lens SM (2014). Cell division: control of the chromosomal passenger complex in time and space. *Chromosoma*, **123**, 25-42.
Van Houdt WJ, Haviv YS, Lu B, et al (2006). The human survivin promoter: a novel transcriptional targeting strategy for treatment of glioma. *J Neurosurg*, **104**, 583-92.
Vanoosthuyse V, Prykhozhij S, Hardwick KG (2007). Shugoshin 2 Regulates Localization of the Chromosomal Passenger proteins in fission yeast mitosis. *Mol Biol Cell*, **18**, 1657-69.
Wang IC, Chen YJ, Hughes D, et al (2005). Forkhead box M1 regulates the transcriptional network of genes essential for mitotic progression and genes encoding the SCF (Skp2-Cks1) ubiquitin ligase. *Mol Cell Biol*, **25**, 10875-94.
Wang R, Lin F, Wang X, et al (2007). The therapeutic potential of survivin promoter-driven siRNA on suppressing tumor growth and enhancing radiosensitivity of human cervical carcinoma cells via downregulating hTERT gene expression. *Cancer Biol Ther*, **6**, 1301-7.
Wang R, Wang X, Li B, et al (2009). Tumor-specific adenovirus-mediated PUMA gene transfer using the survivin promoter enhances radiosensitivity of breast cancer cells *in vitro* and *in vivo*. *Breast Cancer Res TR*, **117**, 45-54.
Wang XJ, Li Y, Huang H, et al (2013). A simple and robust vector-based shRNA expression system used for RNA interference. *PloS one*, **8**, 56110.
Wong R (2011). Apoptosis in cancer: from pathogenesis to treatment. *J Exp Clin Cancer Res*, **30**, 87.
Wu J, Apontes P, Song L, et al (2007). Molecular mechanism of upregulation of survivin transcription by the AT-rich DNA-binding ligand, Hoechst33342: evidence for survivin involvement in drug resistance. *Nucleic acids Res*, **35**, 2390-402.
Xia H, Chen S, Huang H, et al (2015). Survivin over-expression is correlated with a poor prognosis in esophageal cancer patients. *Clin Chim Acta*, **446**, 82-5.
Xiang J, Ouyang Y, Cui Y, et al (2012). Silencing of Notch3 Using shRNA driven by survivin promoter inhibits growth and promotes apoptosis of human T-cell acute lymphoblastic leukemia cells. *Clin Lymphoma Myeloma Leuk*, **12**, 59-65.
Xie Q, Wu Q, Mack S, et al (2015). CDC20 maintains tumor initiating cells. *Oncotarget*, **6**, 13241-54.
Yang CT, Li JM, Weng HH, et al (2010a). Adenovirus-mediated transfer of siRNA against survivin enhances the radiosensitivity of human non-small cell lung cancer cells. *Cancer Gene Ther*, **17**, 120-30.
Yang D, Song X, Zhang J, et al (2010b). Therapeutic potential of siRNA-mediated combined knockdown of the IAP genes (Livin, XIAP, and Survivin) on human bladder cancer T24 cells. *Acta Bioch Bioph Sin*, **42**, 137-44.
Yang L, Cao Z, Li F, et al (2004). Tumor-specific gene expression using the survivin promoter is further increased by hypoxia. *Gene Ther*, **11**, 1215-23.
Yang Y, Gao Y, Chen L, et al (2011). Downregulation of survivin expression and enhanced chemosensitivity of MCF-7 cells to adriamycin by PDMAE/survivin shRNA complex nanoparticles. *Int J Pharm*, **405**, 188-95.
Zhang H-Z, Wang Y, Gao P, et al (2006). Silencing stathmin gene expression by survivin promoter-driven siRNA vector to reverse malignant phenotype of tumor cells. *Cancer Biol Ther*, **5**, 1457-61.
Zhu ZB, Chen Y, Makhija SK, et al (2006a). Survivin promoter-based conditionally replicative adenoviruses target cholangiocarcinoma. *Int J Oncol*, **29**, 1319-29.
Zhu ZB, Makhija SK, Lu B, et al (2004). Transcriptional targeting of tumors with a novel tumor-specific survivin promoter. *Cancer Gene Ther*, **11**, 256-62.
Zhu ZB, Makhija SK, Lu B, et al (2005). Incorporating the survivin promoter in an infectivity enhanced CRAd-analysis of oncolysis and anti-tumor effects *in vitro* and *in vivo*. *Int J Oncol*, **27**, 237-46.
Zhu ZB, Makhija SK, Lu B, et al (2006b). Targeting mesothelioma using an infectivity enhanced survivin-conditionally replicative adenoviruses. *J Thorac Oncol*, **1**, 701.