Therapeutic strategies involving survivin inhibition in cancer

Running title: Survivin inhibition in cancer therapy

David Martínez-García[1,2], Noemí Manero-Rupérez[1,¥], Roberto Quesada[3], Luís Korrodi-Gregório[1]*, Vanessa Soto-Cerrato[1,2]*

[1]Department of Pathology and Experimental Therapeutics, Faculty of Medicine, University of Barcelona, Barcelona, Spain

[2]Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain

[3]Department of Chemistry, Universidad de Burgos, Burgos, Spain

*Corresponding authors:

Dr. Vanessa Soto-Cerrato and Dr. Luís Korrodi-Gregório, University of Barcelona, Pavelló Central, 5a Planta, 5002, C/Feixa Llarga s/n, L'Hospitalet de Llobregat, Barcelona 08907, Spain. E-mail: vsoto@ub.edu; luiskgregorio@gmail.com.

¥ Present address: Hospital del Mar Medical Research Institute, Barcelona Biomedical Research Park, Barcelona, Spain.

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Abstract

Survivin is a small protein that belongs to the inhibitor of apoptosis protein family. It is abundantly expressed in tumors compared to adult differentiated tissues, being associated with poor prognosis in many human neoplasms. This apoptotic inhibitor has a relevant role in both the promotion of cancer cell survival and in the inhibition of cell death. Consequently, aberrant survivin expression stimulates tumor progression and confers resistance to several therapeutic strategies in a variety of tumors. In fact, efficient survivin down-regulation or inhibition results in spontaneous apoptosis or sensitization to chemotherapy and radiotherapy. Therefore, all these features make survivin an attractive therapeutic target to treat cancer. Currently, there are several survivin inhibitors under clinical evaluation, although more specific and efficient survivin inhibitors are being developed. Moreover, novel combination regimens targeting survivin together with other therapeutic approaches are currently being designed and assessed. In this review, recent progress in the therapeutic options targeting survivin for cancer treatment is analyzed. Direct survivin inhibitors and their current development status are explored. Besides this, the major signalling pathways implicated in survivin regulation are described and different therapeutic approaches involving survivin indirect inhibition are evaluated. Finally, promising novel inhibitors under preclinical or clinical evaluation as well as challenges of developing survivin inhibitors as a new therapy for cancer treatment are discussed.

Keywords: survivin inhibitors, smac mimetics, apoptosis, anticancer therapy, chemoresistance, IAPs.

1. Introduction

Cancer is a heterogeneous group of diseases that results not just from aberrant cellular proliferation but also from lack of well-regulated cell death. Resistance to apoptosis is one important evasion mechanism by which tumor cells may present chemoresistance and thus contribute to cancer progression. Consequently, molecules involved in regulation of apoptosis are considered potential targets for cancer therapy. In this regard, survivin, the smallest member of the inhibitor of apoptosis protein (IAP) family, has recently emerged as an attractive drug target due to its dual role in cancer, both in cell cycle progression and apoptosis inhibition. In this review, the molecular mechanisms that regulate survivin gene expression and protein function are summarized, as well as specific survivin inhibitors and other therapeutic strategies involving survivin inhibition are discussed.

1.1. IAPs function and structure

The IAP family embraces a functionally and structurally related group of proteins that function as endogenous cellular inhibitors of apoptosis in response to daily stresses and insults. Due to its relevance as negative regulators of programed cell death, a dysregulation of IAPs is closely related to cancer development and drug resistance [1].

Structure of all mammalian IAPs members contains one to three defining baculovirus IAP repeat (BIR) domains encoding a zinc-finger motif typically arranged at the N-terminus of the protein (Figure 1a). Although the BIR domain is shared by all members of the IAP family, not all BIR-containing proteins exhibit antiapoptotic functions [2]. This domain mediates protein recognition and protein-protein interactions [3]. In addition to the BIR domain, several IAP family members contain a zinc-finger domain called RING (really interesting new gene), which functions as an E3 ubiquitin ligase, an ubiquitin-associated (UBA) domain, a ubiquitin-conjugating (UBC) domain, and/or a caspase-recruitment domain (CARD).

Among all the IAPs, survivin is the smallest member of the IAP family. Encoded by *BIRC5* gene (baculoviral inhibitor of apoptosis repeat-containing 5), mapped to chromosome 17q25, survivin is a 16.5 kDa protein that contains only a single N-terminus BIR domain linked to a C-terminal α-helical coiled coil domain. The latter domain is important for the interaction and formation of the chromosomal passenger complex (CPC), proper segregation of chromosomes and cytokinesis during cell division [4,5]. A dimerization domain at two different locations in the linear sequence of survivin allows it to form a stable homodimer that seems to carry out its mitotic activity, while the monomeric form of survivin is mostly associated with its antiapoptotic activity [6,7] (Figure 1b and 1c). Moreover, different functions of survivin seem to be affected by differential subcellular localization, being the nuclear survivin related to cell division regulation while cytosolic survivin is believed to function as apoptotic suppressor (Figure 2) [8]. Additionally, a small pool of survivin can be found in the mitochondria, from where is rapidly released to the cytosol in response to cell death stimulation and confers resistance to apoptosis [9].

Although the overexpression of survivin inhibits both extrinsic and intrinsic apoptosis pathways contributing to cancer progression, the exact molecular mechanism remains unknown. It has been reported a direct binding of survivin to effector caspases, but a prevailing model is that survivin inhibits apoptosis by interacting with other proteins (Figure 2). One of these interactions is a complex formation between survivin and X-linked inhibitor of apoptosis protein (XIAP) [10]. This IAP-IAP complex enhances XIAP stability against ubiquitin-dependent degradation, increasing in this way the ability of XIAP for inhibiting caspases [11].

Another mechanism proposed is the formation of the complex between survivin and hepatitis B X-interacting protein (HBXIP), which binds to procaspase 9 preventing its recruitment to the apoptosome and later activation [12].

1.2. Survivin as a promising therapeutic target

Due to its dual role both in cell cycle promotion and apoptosis inhibition, survivin has been considered an ideal target for anticancer therapy. Indeed, several molecular approaches that block survivin expression and/or function are emerging as promising therapeutic strategies in cancer [13]. Remarkably, survivin is almost undetectable in most normal differentiated tissues, but prominently expressed in most cancer malignancies [14]. This expression pattern provides selectivity over the tumor cells, thus decreasing the potential side effects in treated patients. Moreover, survivin overexpression has already been correlated with tumor prognosis, being considered a biomarker with a negative correlation on patient clinical outcome and drug resistance in many cancers [15]. Altogether, survivin overexpression in tumors and its key biological roles promoting carcinogenesis and chemoresistance, makes survivin a promising therapeutic target.

2. Pharmacological options targeting survivin

Since survivin has emerged as an ideal target for cancer drug discovery, many survivin inhibitors have been reported in the literature. Some of them are specific direct inhibitors (Figure 2, table 1) but others do not directly bind and interact with survivin protein itself, instead, they usually target other biomolecules and ultimately reduce survivin expression (Figure 3, table 2). In the following sections, we discuss the available information about survivin inhibitors developed so far, as well as the direct or indirect mechanisms by which they repress survivin.

2.1. Survivin direct inhibitors: targeting survivin gene or protein

2.1.1. Transcriptional inhibition of survivin

The inhibition of survivin gene expression is a therapeutic strategy under clinical development with the final aim of counteracting the overexpression of survivin in tumor cells. For this purpose, several small-molecules inhibiting the survivin gene promoter or mRNA have been studied.

YM155 was the first identified small-molecule inhibitor that targets and suppresses specifically the activity of the survivin promoter, regardless of p53 status [16]. Interestingly, it suppressed survivin gene promoter and induced apoptosis in prostate cells as well as promoted tumor regression in human prostate PC3 ectopic xenograft tumors [16]. Other preclinical studies showed the promising anti-cancer properties of YM155 against a panel of 119 human cancer cell lines with an average inhibitory concentration (IC_{50}) of 15 nM. Moreover, continuous 3- or 7-day YM155 infusion (1-10 mg/kg) in xenograft model demonstrated significant antitumor activity without significant toxicity, measured through bodyweight loss [17]. Phase I study reported YM155 as a well-tolerated anticancer drug that showed some efficacy against blood cancers [18]. However, in phase II studies, YM155 showed modest single-agent activity against non-small cell lung carcinoma (NSCLC), but with a disease control rate similar to another second-line agents for advanced NSCLC [19]. The combination of

YM155 with carboplatin and paclitaxel also exhibited a favorable safety profile but failed to demonstrate an improvement in response rate in advanced NSCLC [20].

However, despite having evidence supporting that YM155 can antagonize survivin expression, recent data support that YM155 is a DNA damaging agent where suppression of survivin is a secondary event, probably a consequence of transcriptional repression[21]. Similarly, other study also suggests that inhibition of survivin occurs via suppression of EGFR signaling and its downstream factors [22]. Therefore, YM155 may not be only considered a specific survivin inhibitor.

On the other hand, a recent study identified other compound targeting survivin by high throughput screening of chemical libraries following *in vitro* and *in vivo* analysis [23]. This compound, named FL118, is a nonselective small-molecule inhibitor of survivin expression that structurally resembles the topoisomerase I inhibitor, irinotecan. Its antitumor activity results from inhibiting survivin promoter activity and survivin gene expression. In addition, FL118 also downregulates the expression of myeloid cell leukemia 1 (Mcl-1) and some IAPs, such as XIAP and cellular inhibitor of apoptosis 2 (c-IAP2) [23]. FL118 effectively inhibited cancer cell growth at concentrations lower of 1 nM in a p53 status-independent manner. The *in vivo* studies revealed that FL188 has greater antitumor efficacy without significant toxicity compared with leading first-line chemotherapeutics [23].

In addition to small-molecules targeting survivin gene promoter, antisense oligonucleotides have also been developed to inhibit survivin expression. LY2181308 is one example of single-strand antisense oligonucleotide that targets survivin by binding to and degrading its mRNA preventing its translation into protein and thus, limiting survivin expression. LY2181308 showed significant reduction of both survivin mRNA and protein, as well as cell-cycle arrest, cell-death induction and tumor growth inhibition in several tumor cell lines and human tumor xenografts [24]. Several clinical studies have been carried out showing a favorable safety profile but mixed clinical outcomes [25-27]. As an example, while LY2181308 showed synergistic benefits in patients with refractory or relapsed acute myeloid leukemia when combined with cytarabine and idarubicin [26], no benefit was observed against solid tumors when LY2181308 was used as a single agent or in combination with docetaxel/prednisone [27].

SPC3042 (EZN-3042), another antisense oligonucleotide, was identified as a new agent with higher potency for survivin mRNA inhibition compared to former antisense agents, including LY2181308 [28]. However, SPC3042 not only targets survivin mRNA but also has significant effect over B-cell lymphoma 2 (Bcl-2) mRNA. Downregulation of survivin expression using SPC3042 led to cell cycle arrest, pronounced cellular apoptosis and sensitization of prostate cancer cells to taxol treatment, both *in vitro* and *in vivo*. EZN-3042 showed, as a single agent, a 60% downmodulation of survivin mRNA in tumors of A549 and Calu-6 lung xenograft models and 37-45% of tumor growth inhibition. In addition, when EZN-3042 was combined with paclitaxel, 83% of tumor growth inhibition was achieved [29]. Despite these promising outcomes, phase I trial of EZN-3042 was terminated due its dose-limiting toxicity [30].

Finally, other gene therapy-based approaches are currently being studied, especially in combination with conventional chemotherapeutics. It has recently been demonstrated that small interfering (si) RNA against survivin, combined with temozolomide or etoposide, induced a synergistic cytotoxic effect in glioblastoma cells[31]. Moreover, the combination of the micro RNA miR-542-3p mimic in combination with paclitaxel significantly inhibited *in vivo* tumor growth of HER2-overexpressing breast cancer cells, overcoming their chemoresistance[32].

2.1.2. Protein-protein interaction abrogation

2.1.2.1. Smac mimetics

Second mitochondria-derived activator of caspase (SMAC/DIABLO) is a proapoptotic protein released from mitochondria upon apoptotic stimulation and promotes cytochrome c-dependent apoptosis by binding to and antagonizing IAPs. In this sense, SMAC/DIABLO binds to XIAP releasing caspase-9 from the complex, leading to apoptosis activation. On the other hand, cytosolic survivin is able to bind to SMAC/DIABLO, through its AVPI peptide binding region, inhibiting its pro-apoptotic functions [33]. Furthermore, survivin overexpression can also diminish the pro-apoptotic functions of SMAC/DIABLO by delaying its release from mitochondria through direct union after apoptotic stimuli [34].

Withanone, a natural product derived from roots of *Withania somnifera*, was studied as a possible competitor of SMAC/DIABLO for its binding site in survivin protein. Computational docking analysis showed that withanone binds to survivin BIR domain in the same hydrophobic cavity as that of SMAC/DIABLO, therefore being able to interfere with its inhibitory activity against caspases [35]. Although the anticancer properties of withanone have already been studied in several cancer cell lines [36], experimental analysis are needed to confirm whether this compound specifically binds and inhibits survivin. Similarly, analogs of the phenolic component of black pepper piperine have also been described as potential survivin inhibitors by binding to the hydrophobic cavity of the BIR domain [37]. Piperine was capable to inhibit cell growth and induce apoptosis in several types of cancer cells, such as human colon cancer cells [38], and suppressed tumor growth and metastasis in mice models, but more studies are needed to determine whether its anticancer potential is mediated through survivin inhibition.

In a more recent work, using similarity-based virtual screening for the AVPI peptide in the survivin-SMAC crystal complex, UC-112 was identified as a potent and selective survivin inhibitor [39]. UC-112 showed potent cell growth inhibition in human melanoma and prostate cancer cell lines, as well as antitumor activity in *in vivo* studies. Furthermore, survivin levels were strongly downregulated upon UC-112 treatment *in vitro* and *in vivo*. Based on the UC-112 scaffold, new survivin inhibitors, being the most potent ones the 4g and 10f, were designed and synthesized [40,41]. Compared to UC-112, its analog 4g showed an activity improvement of four folds in growth inhibition of cancer cell lines. Moreover, both compounds maintained the high selectivity for survivin showed by its parent compound UC-112. *In vivo* studies also showed an effective suppression of tumor growth and strong induction of cancer cell apoptosis in tumor tissues.

All this data, along with the promising outcomes given by other IAP-specific SMAC mimetics [42], which are under evaluation in early clinical trials both as monotherapy or in rational combination therapies, encourage the study of compounds with this mechanism of action.

2.1.2.2. Hsp90-survivin inhibitors

Another well-studied protein interaction is the association between heat shock protein 90 (Hsp90) and survivin. Hsp90 directly binds, through its N domain, to the BIR domain of survivin [43]. Besides this, it has been studied how the chaperone function of Hsp90 is required to preserve survivin stability *in vivo* and disruption of this complex by using Hps90 inhibitors triggers loss of survivin via proteasomal-dependent degradation. Blockage of survivin-Hsp90 complex formation promoted apoptosis and mitotic defects in cultured cells. Hence, molecular

antagonists of survivin-Hsp90 interaction may provide another rational approach to treat cancer.
Shepherdine, a small peptidomimetic, was rationally designed for this purpose [44]. Shepherdine makes extensive contact with the N domain of Hsp90, thus destabilizing survivin and inducing massive death of tumor cells by apoptotic and nonapoptotic mechanisms. Shepherdine not only affects survivin expression but also destabilized other Hsp90 client proteins, however, it has been proved its good selectivity, because it does not affect normal cells while maintaining excellent antitumor activity. Systemic administration of shepherdine *in vivo* efficiently inhibited human tumor growth in mice without showing significant toxicity [44].
Based on shepherdine, a non-peptidic small molecule called AICAR (5-aminoimidazole-4-carboxamide ribonucleotide) has been identified, through structure- and dynamics-based rational design, as a new Hsp90 inhibitor [45]. AICAR showed the capability of directly binding to the N-terminal domain of Hsp90 and destabilize its clients proteins, including survivin. Experimental tests showed that AICAR exhibits antiproliferative and proapoptotic activity in melanoma, prostate and cervical cancer cell lines, while not affecting proliferation of normal human fibroblasts [45].

2.1.2.3. Survivin homodimerization inhibitors

It is known that monomeric and dimeric forms of survivin coexist, being the homodimeric form more related with the promotion of mitosis by enhancing tubulin stability [6]. Thus, development of specific inhibitors targeting the dimerization site of survivin may be a feasible approach to treat cancer. This strategy becomes more attractive due to studies that showed how exposition of the hydrophobic interface of a dimeric protein often cause conformational changes, which leads to destabilization and degradation of the protein [46]. Furthermore, homodimerization interface of survivin is not shared with other IAPs, therefore inhibitors of this site may increase their selectivity against survivin.

Abbott 8 was identified using NMR and affinity-based screening as a small soluble compound that binds to the dimer interface of survivin [47]. Several analogues were further developed reaching compounds with nanomolar affinities, however, more studies are needed to evaluate their anticancer activity.

In this context, using *in silico* screening targeting the critical dimerization core residues of survivin, LQZ-7 was identified [48]. LQZ-7 was able to dissociate the dimeric survivin into monomers *in vitro* and to promote its proteasome-dependent degradation in cells. Further analysis of LQZ-7 analogs led to the identification of LQZ-7F, a compound able to disrupt more effectively survivin dimerization, cause proteasome-dependent degradation, mitotic arrest and inhibit cancer cell survival through induction of spontaneous apoptosis. LQZ-7F was also effective in suppressing PC3 xenograft tumor growth and reducing survivin levels, without showing significant toxicity in mice after multiple dosing[48].

2.1.2.4. Mitosis-related proteins inhibitors

Survivin plays an essential role in cell mitosis, including chromosome segregation and cytokinesis, mostly as an integral component of CPC [49]. It is well documented how depletion of survivin causes cell proliferation arrest, sustained prometaphase blockade, chromosomal defects and cytokinesis failure [50]. Additionally, a distinct pool of subcellular survivin is

associated with polymerized microtubules, sustaining their stability, thereby contributing to the bipolar spindle assembly [50].

In this context and using *in silico* analysis, several hotspot residues related to protein-protein interaction were found in survivin, including its CPC complex interface [51]. Therefore, a pharmacophore model was derived and used to virtually screen databases of compounds. Using this methodology, indinavir, a protease inhibitor approved for the treatment of human immunodeficiency virus (HIV), was identified as an inhibitor of the interaction of survivin with its binding partners, such as CPC complex. Indinavir was able to impart anti-proliferative and apoptotic activity in breast cancer cells, decreasing Aurora B and XIAP proteins and inducing caspase-3 activation. Although preliminary biochemical results indicate that indinavir could inhibit both survivin-Aurora B in the CPC, and survivin-XIAP interactions, further investigations are needed to understand the antiproliferative mechanism of action of this compound.

In another *in silico* study, S12, a small molecule that targets a specific cavity adjacent to the survivin dimerization surfaces, was identified [52]. S12 showed disruption of the spindle formation, which led to mitotic arrest of cancer cells, followed by cell death with no apparent toxic effect over non-proliferating and normal resting cells. Moreover, *in vivo* studies showed how S12 was able to inhibit tumor growth without producing systemic toxicity in animals [52].

Finally, the importance of the protein complex formation between survivin and the small GTPase Ran (Ras-related nuclear protein) has also been studied. Ran is implicated in microtubule stability and mitotic spindle assembly in tumor cells [53]. The small-molecule LLP-3, derived from Abbott 8 compound, showed an effective disruption of the survivin-Ran complex, leading to survival and growth inhibition of glioma stem cells, both *in vitro* and *in vivo* [54].

2.2. Survivin indirect inhibitors: targeting key cellular signaling pathways

It has already been shown that survivin expression, either through down or up-regulation, could be triggered by several signaling pathways. The most actively described are shown in Figure 3 and will be discussed below.

2.2.1. Cell growth, proliferation and survival

2.2.1.1. PI3K/AKT

The phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/protein kinase B (PKB or AKT) signaling pathway is involved in several cellular functions including cell proliferation, growth and survival. The tyrosine receptor activation, via external signals, leads to class I PI3K recruitment to the plasma membrane and consequent activation to unleash a cascade of phosphorylations that will result in AKT activation. The AKT pathway has abundant downstream components controlling several cellular processes and is inappropriately activated in many cancers. Constitutive activation of PI3K receptor and somatic mutations in any component of the pathway or its negative regulators were observed in many cancers [55]. During normal angiogenesis, the activation of PI3K/AKT pathway through upstream stimulation of vascular endothelial growth factor (VEGF)/vascular endothelial growth factor receptor (VEGFR) or angiopoietin 1 (Ang1)/angiopoietin receptor (Tie2), leads to an increase in survivin expression. Besides this, insulin growth factor (IGF-1)/PI3K/Akt/mammalian target of rapamycin (mTOR) signaling elevates survivin levels in prostate cancer cells via rapid changes in mRNA translation[56]. Likewise, several receptors (e.g. Granulocyte-macrophage colony-stimulating factor receptor(GM-CSFR), epidermal growth factor receptor (EGFR) and human

epidermal growth factor receptor 2 and 3 (HER-2 and HER-3) upregulate survivin levels through this route in cancer [32,57].

Particularly, PI3K/AKT signaling is a potent regulator of survivin expression through different mechanisms: 1) activation of mTOR/Ribosomal protein S6 kinase beta-1 (p70S6K) axis induces the translation of various oncogenic proteins such as hypoxia-inducible factor 1-alpha (HIF-1α), which in turn control cell survival related genes such as survivin (upregulation) [58]; 2) Mouse double minute 2 homolog (MDM2) negatively regulates the tumor suppressor p53, which decreases survivin expression via direct binding to the promoter [59]; 3) AKT negatively regulates the transcription factors forkhead box protein O1 (FOXO1) and O3a (FOXO3a) that physically associate with the survivin promoter, repressing its expression [60] and 4) Nuclear factor kappa B (NF-κB), which is regulated upstream by the AKT/inhibitor of NF-κB kinase (IKK) axis, is associated with the transcriptional upregulation of survivin [61,62].

Accordingly, inhibiting this pathway has become an important strategy in cancer treatment. In breast cancer, herceptin and lapatinib (HER-2 inhibitors) and AG1478 (EGFR inhibitor) inhibit survivin expression [63-65]. In ovarian cancer, similar results were observed with the EGFR inhibitors gefitinib and PD153035 [66,67]. However, most reports have studied the inhibition of PI3K through LY294002 and wortmannin in several types of cancer: breast, colon, liver, ovary, lung and in leukemia [60,65,67-72]. Concerning the inhibition of AKT, MK-2206 strongly blocks survivin in glioma and colon cancer [73]. Moreover, SH5 inhibitor significantly reduces survivin levels in lung cancer [68] and similar results were observed with AKT inhibitor X in prostate cancer [69]. Likewise, mTOR inhibitors, more specifically rapamycin, firmly downregulate survivin in glioblastoma, multiple myeloma and prostate cancer [58,74]. The induction of survivin was also inhibited by the NF-κB inhibitors, SN50 and BAY 11-7082 in endothelial cells [61,75]. Additionally, downregulation of survivin was observed with panepoxydone in breast cancer, and this seems to be related with NF-κB inhibition [76]. Cyclooxygenase-2 (COX-2) induces survivin expression through AKT activation in several cancers (e.g. glioblastoma, lymphoma, myeloma, breast, colon and prostate) and this action could be reversed through its inhibitors, celecoxib and etodolac [77].

2.2.1.2. JAK/STAT

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway plays a critical role in cell proliferation, differentiation, migration, apoptosis and immunity [78]. A wide range of cytokines, hormones and growth factors, and their respective receptors stimulate the JAK/STAT pathway [78]. JAK activation results in the recruitment and activation of STAT proteins that will then translocate to the nucleus and induce the transcription of target genes, such as survivin [78]. It is known that dysregulation of JAK/STAT pathway participates in cancer development and metastasis [78].

Interleukin 6 (IL-6), induce STAT3 activation leading to an increase on survivin expression in breast cancer cells [79]. In leukemia, the up-regulation of survivin involving the JAK/STAT signaling pathway is dependent on GM-CSF [80] and breakpoint cluster region protein (BCR)-Abelson murine leukemia viral oncogene homolog (ABL) [81].

Interestingly, after blocking JAK2 kinase with the TG101209 inhibitor, a decrease of survivin is observed in leukemia [81]. Another JAK2 inhibitor known as AG490 [82] has shown to downregulate survivin in lymphoma as well as leukemia. Similar results were reported with the JAK2 inhibitor SD-1029 for breast and ovary cancers [83]. Our group has also shown that novel indol-based

tambjamine analogues were able to reduce survivin levels by inhibiting JAK/STAT pathway through an unknown mechanism of action [84 and unpublished data].

2.2.1.3. MAPK/ERK

Mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling mediates cellular proliferation, differentiation, survival, development, migration and apoptosis. Ligand binding to the tyrosine kinase receptor causes the dimerization and phosphorylation of its cytoplasmic domains. Adaptor proteins bind to the receptor, triggering Rat sarcoma (RAS) GTPase, which in turn activates a cascade of kinases: MAPKKK (Raf, rapid accelerated fibrosarcoma), MAPKK (MEK, mitogen-activated protein kinase kinase)) and MAPK (ERK). Activated ERK is transported to the nucleus where phosphorylates several transcription factors regulating several target genes including survivin. MAPK/ERK signaling is involved in cancer progression, particularly in proliferation and immune escape and numerous mutations have been identified in tumors, such as in RAS genes [55].

Epidermal growth factor (EGF) induces the activation of MAPK/ERK signaling and increases survivin levels [85]. Cytokines such as Granulocyte-macrophage colony-stimulating (GM-CSF) and the BCR-ABL tyrosine kinase could induce survivin expression via MAPK/ERK dependent mechanism in leukemia [86]. Moreover, HER-2 up-regulation of survivin is regulated by MAPK/ERK signaling, apart from the PI3K/AKT contribution in breast cancer [70]. Similar results were observed with c-Harvey rat sarcoma viral oncogene homolog (c-H-RAS), where both MAPK/ERK and PI3K/AKT are participating in survivin overexpression [72].

Interestingly, MEK inhibitors significantly reduce survivin levels. In particular, PD98059 in breast cancer [65] and leukemia, and similar results were described with CI1040 in leukemia [86]. Besides this, PD0325901 (a CI1040 derivative) prevented the placental lactogen induction of survivin [66]. The U0126 inhibitor strongly blocks MAPK induction of survivin in leukemia [86], breast cancer [70] and keratinocytes [72]. In addition, BCR-ABL inhibition by imatinib significantly hampers MAPK/ERK survivin expression in leukemia cells [86].

2.2.2. Cell cycle regulation

Survivin is expressed in a cell cycle-dependent way and localizes in the mitotic apparatus. The cyclin-dependent kinases (CDKs) 2/4 are active in both G1 and S phases and phosphorylate retinoblastoma (Rb) relieving it from the E2F transcription factors to allow the cell cycle transition. Survivin is positively regulated by E2F1, E2F2 and E2F3 through cell cycle-dependent element (CDE)/cell cycle genes homology region (CHR) dependent mechanism while is negatively regulated by E2F4 and E2F5 repressors [87]. Moreover, the CDK1/cyclin-B1 pair phosphorylates survivin on Thr34, contributing to its stability and its association with caspases [88].

Some CDKs inhibitors, in addition to arresting the cell cycle, have an interesting ability to induce apoptosis. In addition, CDK inhibitors abrogate survivin phosphorylation on Thr34 leading to its degradation [89]. The specific CDKs inhibitor (purvalanol A) downregulates survivin through JAK/STAT inhibition in gastric cancer [79]. A broad spectrum CDK inhibitor known as flavopiridol inhibits survivin phosphorylation on Thr34, reducing its protein levels in cervical cancer and has shown its antitumor activity in several phase II clinical trials [90,91]. The specific CDK2 inhibitor NU6140 also diminishes survivin expression in cervical and ovary cancers [88]. Finally, Cephalochromin is a selective bacterial inhibitor that reduces the levels of CDK2/cyclin-

E and CDK4/cyclin-D1 pairs producing cell cycle arrest (G_0/G_1 phase) and consequently decreasing survivin expression and inducing apoptosis [92].

2.2.3. Cellular stress: p38MAPK

The p38MAPK signaling is involved in inflammation, cell cycle, cell death, development, differentiation and senescence [93]. Environmental stresses (e.g. UV and heat) and inflammatory cytokines trigger this pathway by phosphorylation of MAPKKKs. These kinases signal the cascade through MKK3 and MKK6 and finally p38MAPK which will finally activate transcription factors (e.g. p53) that will regulate several target gene expression [93].

Activation of the p38MAPK route by the COX-2 inhibitor celecoxib induces survivin downregulation in colon cancer cells [94]. Likewise, oxaliplatin, a chemotherapeutic drug, leads to colon cancer cell death through survivin downregulation via p38MAPK pathway and via proteasomal degradation [95].

2.2.4. Development and Differentiation

2.2.4.1. TGFβ

Transforming growth factor beta (TGF-β) signaling controls several cellular functions including cell growth, proliferation, differentiation, development and migration. Upon ligand binding, constitutively active TGF-β receptor II (TβRII) recruits and activates by phosphorylation the TβRI. Due to this activity, Smad proteins, Smad2 and Smad3, are phosphorylated and form a complex with co-Smad (Smad4) in the cytoplasm, which is then translocated to the nucleus where it regulates gene expression, including survivin [96]. Although TGFβ is an important tumor suppressor, cancer cells subvert this pathway by taking control of the tumor promoting arm [96]. Interestingly, an overexpression of TGF-β1 was described in various types of cancer. In addition, inactivating mutations on SMAD2 and SMAD4 were reported in hepatocellular, colorectal and lung cancer [96].

The activation of the TGF-β pathway in colon cancer causes the hypophosphorylation of Rb via Smad3-dependent mechanism, which causes the association of Rb/E2F4 repressive complex to CDE/CHR elements of survivin gene promoting its downregulation [71,97]. Similarly, TGF-β inhibition of PI3K/AKT signaling through protein kinase A (PKA)/A-kinase anchoring protein (AKAP149)/protein phosphatase 2 (PP2A) may also reduce the survivin levels [71,97]. Furthermore, TGFβ activation of PKA, leads also to the phosphorylation of survivin in Ser20 inducing its degradation [97].

2.2.4.2. Wnt/β-Catenin

Wnt signaling regulates stem cell pluripotency, differentiation and embryonic development. In absence of Wnt signaling, Adenomatous polyposis coli (APC)/Axin/glycogen synthase kinase 3 beta (GSK-3β) inhibitory complex leads to the ubiquitination and degradation of β-catenin. Wnt receptors activation hampers this complex, displacing Axin and inhibiting GSK-3β activity. This leads to β-catenin accumulation in cytoplasm and its translocation to the nucleus where it binds to Lymphoid enhancer-binding factor (LEF)/TCF transcription factors and co-activators (e.g. CREB-binding protein, CBP) regulating a variety of genes, including survivin [98].

A variety of Wnt and APC mutations and alterations are frequently observed in cancer [98]. It has been reported that TCF/β-catenin induces survivin expression in lung, colorectal and breast cancers [63,98,99].

Drugs binding to HER-2 receptor, such as Herceptin (trastuzumab) in breast cancer, compromise β-catenin stabilization and consequently decrease survivin expression [63]. Furthermore, an antibody against Wnt2 protein, Wnt-2 Ab, induces apoptosis inhibiting this process in lung cancer [98]. Downregulation of β-catenin and survivin expression by ICG-001 (a β-catenin/CBP disruptor) was observed in colon cancer [100].

2.2.4.3. Notch

Notch signaling is involved in important cellular processes such as proliferation, development, differentiation and homeostasis [101]. It mediates cell-cell communication through interaction with transmembrane ligands on adjacent cells. Ligand binding results in Notch receptor cleavage through γ-secretase releasing the notch intracellular domain (NICD) that is then translocated to the nucleus where it modifies DNA transcription. Particularly, NICD associates with CBF1/Su(H)/Lag1 (CSL) transcription factor in order to activate Notch target genes, including survivin [101,102]. Hypoxia and Jagged-1 ligand activate Notch signaling increasing survivin expression in lung cancer [103]. In addition, HIF-1α promotes Notch induction of survivin, suggesting that HIF1-α interacts with NICD facilitating the CSL transcription factor association with a RBP-Jκ site in the survivin promoter [102].

Aberrant Notch signaling has been associated with tumorigenesis and cancer progression in lung, pancreas and breast cancer models. In fact, mutations on Notch receptors and consequently nuclear overexpression of NICD were described in leukemia and lymphoma [101].

Under hypoxia, both Echinomycin (HIF-1α inhibitor) and inhibitors of γ-secretase (MRK-003 and a specific peptide inhibitor), decrease survivin expression in lung and breast cancers [102].

2.2.5. Others

Survivin promoter has several sites for the transcription factor Sp1 and mutations on some of those sites reduce survivin expression by 60-80% in cervical cancer [104,105]. In contrast, Sp1 could also recruit transcriptional repressors such as p53, DNMT1 and HDAC and consequently suppress survivin promoter activity [106]. Interestingly, Sp1 inhibition by mithramycin A or oxaliplatin significantly decreased survivin promoter activity in colon and esophageal cancers [106,107]. Moreover, the small molecule terameprocol significantly decreased survivin levels by hampering Sp1 binding to survivin promoter and through the downregulation of CDK1 that leads to survivin degradation. This effect was observed in colorectal, prostate, hepatic, erythroleukemia and breast cancers [108].

Survivin has yet binding sites to other transcription factors as we have mentioned previously. Several of these transcription factors are important survivin inductors, like the GATA-1 [109], c-myc [110] and DEC1 [111] transcription factors. KLF5 binds directly to survivin promoter on three specific sites inducing its expression, but could also interact with p53 and block its repression on survivin promoter [112]. In addition, it has been reported that survivin is targeted by GLI2 transcription factor, a Hedgehog signaling effector. Defective activation of hedgehog signaling has been described in many cancers. 11 GLI binding sites were found in survivin promoter. Inhibiting Hedgehog signaling through SMO inhibitor cyclopamine strongly reduces survivin promoter activity in melanoma [105]. Besides, an specific inhibitor of GLI known as GANT61 also significantly inhibits survivin promoter activity and consequently reduces survivin protein in melanoma, colon, lung, pancreatic and adrenal gland cancers [105]. Furthermore, palmatine has recently been described to inhibit GLI/collagen type 1 alpha 1 (COL1A1) in stellate cells and survivin in cancer cells [113].

Other molecules have also shown to reduce survivin levels in several cancers: an inhibitor of CRM1 (KPT-185), a protein that plays a critical role in the nuclear export of proteins; SF002-96-1 (natural lactone) through indirect inhibition of transcription factors, STAT3 and NF-κB [114] and several small molecules (e.g. GDP366, the HIV protease inhibitor Nelfinavir and the derivative of retinoic acid tretinoin) through an unknown mechanism of action [115-117].

Concluding Remarks and future perspectives

Many preclinical studies have extensively demonstrated that survivin has a relevant role in promoting tumor growth as well as inducing resistance to chemotherapy in several neoplasms. Moreover, survivin shows a differential expression, being preferentially and abundantly expressed in tumors but not in adult differentiated tissues. Altogether, these features make survivin a promising therapeutic target to treat cancer. However, early clinical data indicates that the first direct inhibitors of survivin available show modest activity as single agents and, in some cases, dose-limiting toxicities. These are the cases of LY2181308 oligonucleotide or the small-molecule inhibitor YM155. This limited response may be due to deficiencies in complete and selective survivin inhibition in patients and to tumor heterogeneity. Therefore, inhibitors with more specific mechanisms targeting survivin, such as smac mimetics or survivin homodimerization inhibitors, are expected to show better clinical results. On the other hand, several indirect survivin inhibitors are currently in clinical trials, such as flavopiridol or lapatinib, although their effects may be due to the combination of survivin inhibition together with other mechanisms of action [64,91]. Additionally, efforts directed at identification of specific biomarkers useful for the selection of patients benefiting from the treatment should be made.

Immunotherapy is another therapeutic approach under current evaluation. It is focused on targeting survivin through the stimulation of the immune system to provoke a direct response against the tumor. Some clinical trials, like vaccination with survivin-2B80-88 peptide [118], have shown limited clinical responses. On the other hand, the good tolerability demonstrated offer promise to optimize this therapeutic approach. Indeed, there is some expectation in several phase I and II clinical trials using survivin vaccines (NCT03029403, NCT03349450, among others), which are currently recruiting patients to evaluate its safety, tolerability and effectiveness in combination with other treatments.

Finally, it is anticipated that the maximum therapeutic effect of survivin inhibitors will be completely achieved in combination regimens. Since targeting survivin sensitizes cancer cells to apoptosis [119], it is expected that survivin inhibitors will enhance the apoptotic effect induced by traditional chemotherapeutic drugs or radiotherapy, showing synergistic effects and overcoming the treatment resistance observed in some patients. Several studies combining chemotherapeutics with chemical survivin inhibitors have already demonstrated this ability [86,91] and some others are currently under evaluation. This is also the case for novel gene therapy-based approaches targeting survivin, when administered in combination with chemotherapeutics. Preliminary studies have demonstrated that these combinations are able to enhance cancer cells sensitivity to chemotherapeutics [31] and overcome chemoresistance in certain types of cancers [32].

The reviewed results underscore survivin as an attractive and promising cancer drug target. Future efforts may be focused on the development of synergistic combinations between direct or indirect survivin inhibitors, or gene therapy-based approaches, together with chemotherapeutic drugs in order to efficiently treat cancer.

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Author biosketches

Vanessa Soto-Cerrato obtained her bachelor's degree in Biology in 2001, her master's degree in Biomedical Sciences in 2002 and a Ph.D. in Cellular Biology and Pathology in 2007, at the University of Barcelona. During those periods, she worked in Molecular Genetics (Molecular Biology Institute, University of Copenhagen, Denmark) and in Cancer Experimental Therapeutics (School of Medicine, University of Barcelona). Then she joined Ferrer International Pharmaceuticals as a postdoctoral fellow and was involved in Drug Discovery Programs for Oncology, Neurology and Regenerative Medicine. She is currently an Associate Professor at the School of Medicine at the University of Barcelona, where she has been

involved in different projects regarding the identification and preclinical development of novel proapoptotic anticancer drugs for different types of cancers.

Luís Korrodi-Gregório obtained his B.Sc. degree in Biology from the University of Aveiro, Portugal in 2007 and his Ph.D. degree in Biochemistry from the University of Aveiro in 2012 under the supervision of Prof. Edgar da Cruz e Silva and Prof. Margarida Fardilha. In 2013 he joined as a postdoctoral researcher the Cancer Cell Biology Group under Prof. Ricardo Pérez-Tomás's supervision at the University of Barcelona, Spain, and his research was focused in drug screening and discovering the most promising mechanism of action to tackle cellular internal pH and apoptosis.

Roberto Quesada obtained his Ph.D. degree in Chemistry at the University of Oviedo in 2002. After postdoctoral appointments at the Trinity College Dublin and the University of Southampton, he moved to the University of Burgos in 2008 as ''Ramon y Cajal'' Fellow and became Associate Professor in 2012. His research interests include synthetic and supramolecular chemistry, with a focus in biological applications.

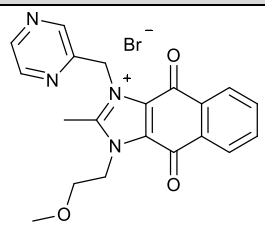
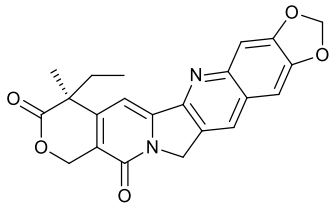
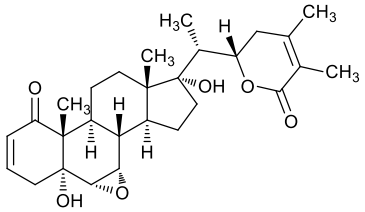
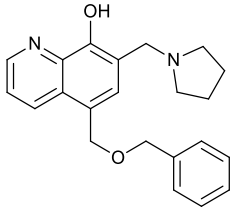
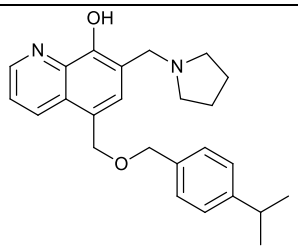
David Martínez-García obtained his bachelor's degree in Biomedical Science and his master's degree in Basic and Translational Research in Cancer at the University of Barcelona in 2014 and 2015, respectively. He is currently pursuing a Ph.D. degree in Medicine and Translational Research under the supervision of Dra. Vanessa Soto-Cerrato and Dr. Ricardo Pérez-Tomás. He is working on the study of survivin in tumor progression and chemoresistance in human lung cancer and he is focused on the design and preclinical development of new inhibitors that effectively block survivin, and therefore the progression of this neoplasm, with minimal side effects.

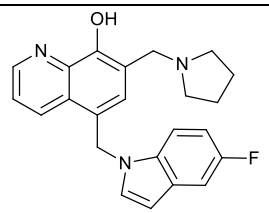
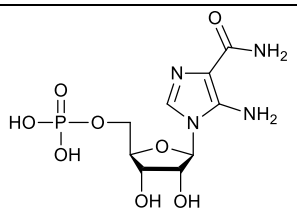
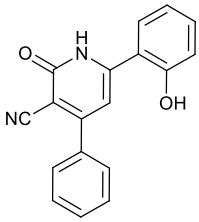
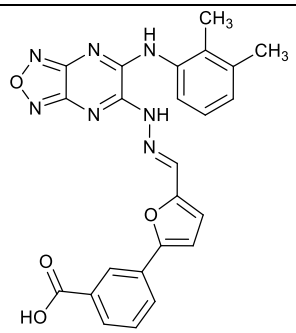
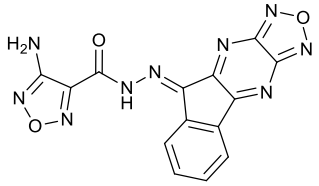
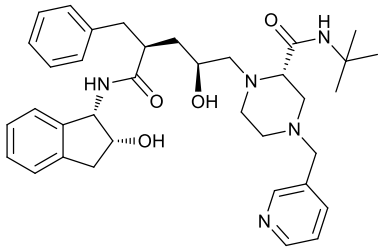
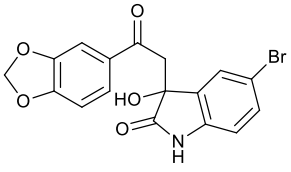
Noemí Manero Rupérez obtained her bachelor's degree in Biomedicine in 2016 and her Master´s degree in Basic and Translational Research in Cancer in 2017 at the University of Barcelona, Spain. In the Cancer Cell Biology Research Group, her research was focused on studying the mechanism of action of several novel anticancer and proapoptotic compounds. She is currently pursuing a Ph.D. degree at the Hospital del Mar Medical Research Institute mainly focused on novel drug targets to treat cancer.

Tables

Table 1. Direct survivin inhibitors.

Mechanism of	Name	Structure	Clinical	Reference

action			trials	
Transcriptional inhibition	YM155		Phase II	16-20
	FL118			23
mRNA inhibition	LY2181308	5’-TGTGCTATTCTGTGAATT-3’	Phase II	24-27
	SPC3042/ EZN-3042	5’-CTCAatccatggCAGc–3’	Phase I	28-30
SMAC mimetics	Withanone			35,36
	UC-112			39
	4g			40

	10f			41
Survivin-Hsp90 complex abrogation	Sepherdine	KHSSGCAFL		44
	AICAR			45
Survivin homodimerization inhibition	Abbott 8			47
	LQZ-7			48
	LQZ-7F			48
Survivin-Mitosis-related proteins inhibition	Indinavir		Phase II	51
	S12			52

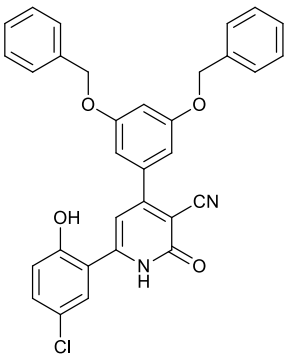
	LLP-3			54

Table 2. Indirect survivin inhibitors.

Biological Process	Pathway	№	Name	Target	References
Cell growth, proliferation and survival	PI3K/AKT	1	Herceptin	HER-2	63
		2	Lapatinib	HER-2	64
		3	AG1478	EGFR	65
		4	Gefitinib	EGFR	66
		5	PD153035	EGFR	67
		6	LY294002	PI3K	57,60,64,65,67-72,75
		7	Wortmannin	PI3K	68,72
		8	MKK-2206	AKT	73
		9	SH5	AKT	68
		10	AKT inhibitor X	AKT	69
		11	Rapamycin	mTOR	57,58,74
		12	SN50	NFκB	75
		13	BAY 11-7082	NFκB	61
		14	Panepoxydone	NFκB	76
		15	Celecoxib	COX-2	77
		16	Etodolac	COX-2	77
	JAK/STAT	17	TG101209	JAK2	81
		18	AG490	JAK2	82

		19	SD-1029	JAK2	[83]
	MAPK/ERK	20	PD98059	MEK	[65]
		21	Cl1040	MEK	[86]
		22	PD0325901	MEK	[66]
		23	U0126	MEK	[70,72,85,86]
		24	Imatinib	BCR-ABL	[86]
Cell cycle regulation	Cell cycle	25	Purvalanol A	CDK1/2/4	[90,120]
		26	Flavopiridol	CDK1/2/4/6	[90]
		27	NU6140	CDK2	[88]
		28	Cephalochromin	CDK2/4	[92]
Cellular stress	p38 pathway	15	Celecoxib	COX-2	[77,94]
		29	Oxaliplatin	DNA synthesis	[77,95]
Development and differentiation	TGF-beta pathway	30	TGF-beta	TGF-beta receptor	[71,97]
	Wnt/β-catenin	31	Wnt-2 Ab	Frizzled Receptors	[98]
		32	ICG-001	β-catenin/CBP	[100]
	Notch	33	Echynomicin	HIF-1α	[102]
		34	Z-Leu-Leu-Nle-CHO peptide	γ-secretase	[102]
		35	MRK-003	γ-secretase	[102]

Correlative numbers correspond to those depicted in figure 3.

Figure Legends

Figure 1. a. Primary structure of IAP proteins in mammals. The main domains are shown in different colors. **b.** Structure of survivin protein. **c.** Structure of dimeric survivin protein.

Figure 2. Survivin sub-cellular functions and direct survivin inhibitors. The main mechanisms of action of direct survivin inhibitors are shown. **1- Transcriptional inhibitors** decrease survivin expression levels; **2-Smac mimetics** impede that survivin inhibit caspases; **3-Hsp90 inhibitors** destabilize the Hsp90-survivin complex, inducing survivin degradation. **4- Homodimerization inhibitors** disassemble survivin homodimer, increasing survivin degradation. **5- Mitotic inhibitors** prevent CPC complex formation and survivin interaction with other mitosis-related proteins.

Figure 3. Signaling pathways that regulate survivin expression and their corresponding indirect survivin inhibitors. Red numbers correspond to those inhibitors listed in table 2.

Figure 1.

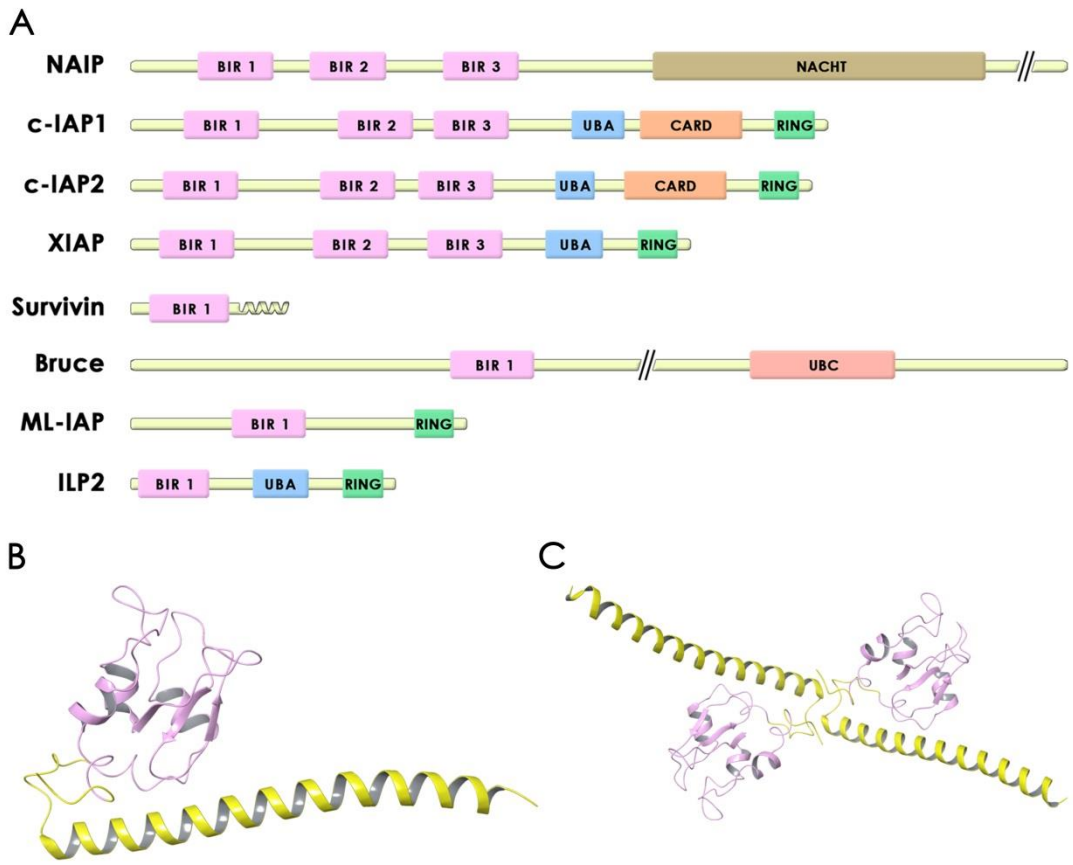

A
NAIP
BIR 1
BIR 2
BIR 3
NACHT
c-IAP1
BIR 1
BIR 2
BIR 3
UBA
CARD
RING
c-IAP2
BIR 1
BIR 2
BIR 3
UBA
CARD
RING
XIAP
BIR 1
BIR 2
BIR 3
UBA
RING
Survivin
BIR 1
Bruce
BIR 1
UBC
ML-IAP
BIR 1
RING
ILP2
BIR 1
UBA
RING
B
C

Figure 2.

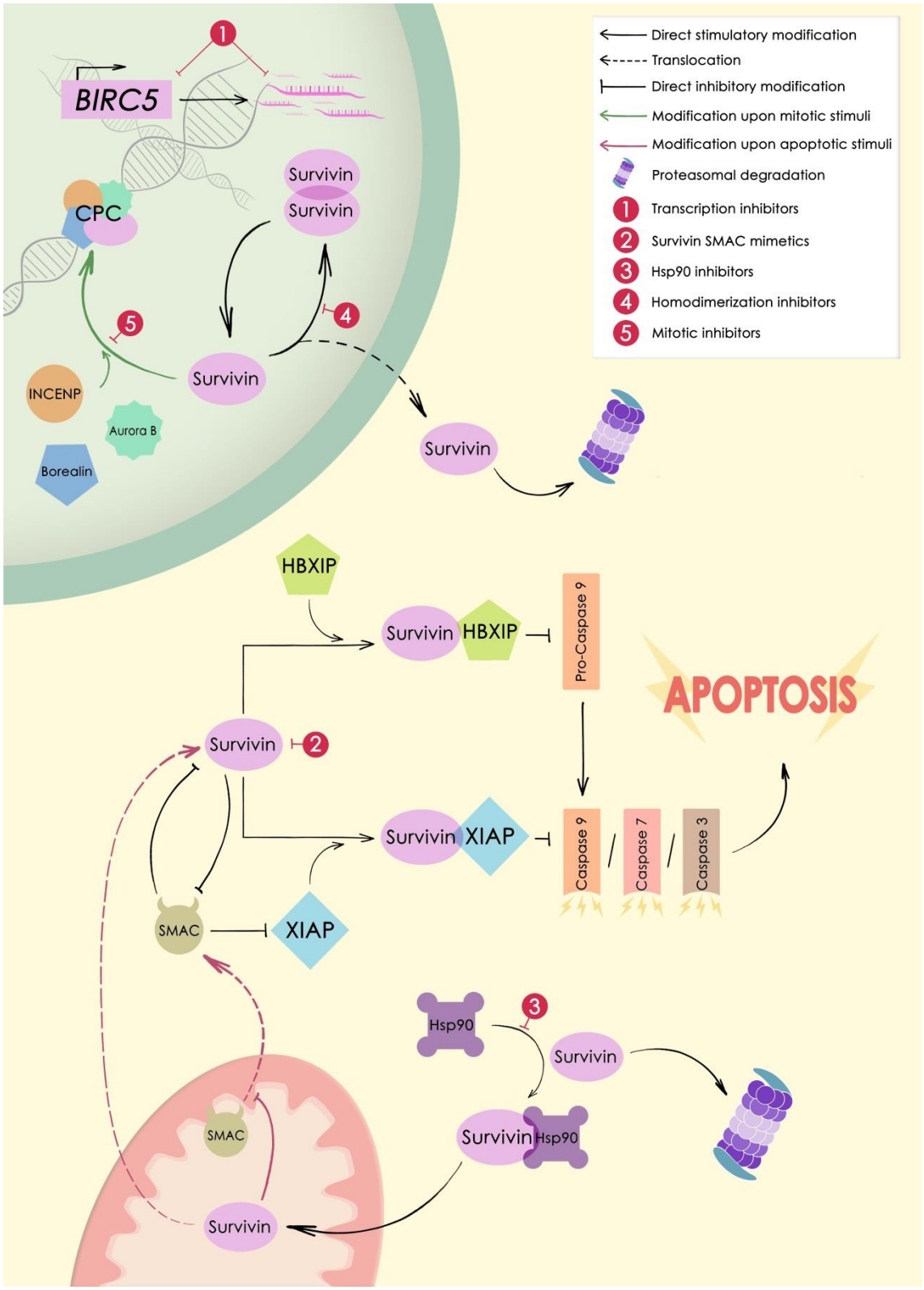
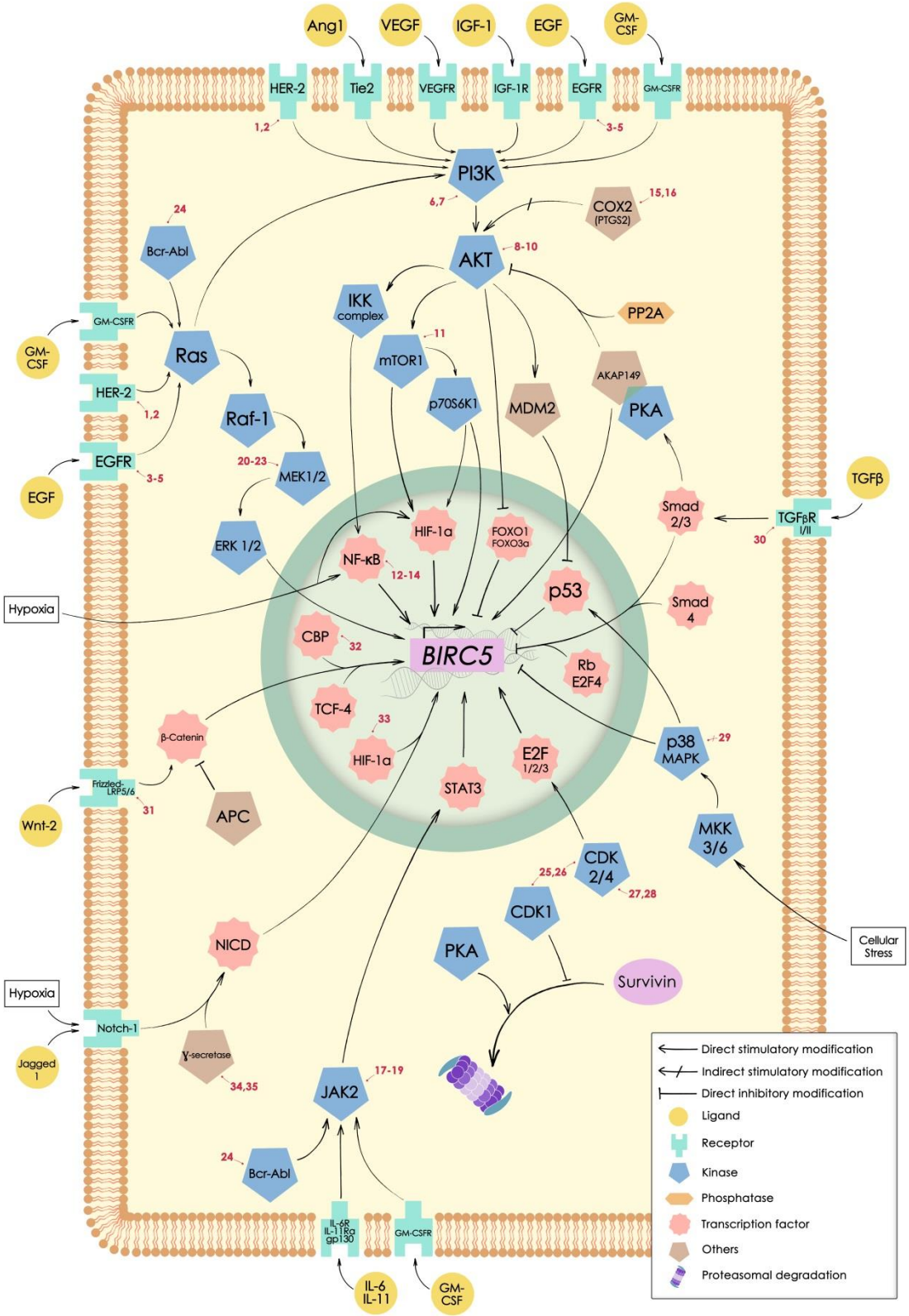

Direct stimulatory modification
Translocation
Direct inhibitory modification
Modification upon mitotic stimuli
Modification upon apoptotic stimuli
Proteasomal degradation
1 Transcription inhibitors
2 Survivin SMAC mimetics
3 Hsp90 inhibitors
4 Homodimerization inhibitors
5 Mitotic inhibitors
BIRC5
CPC
Survivin
Survivin
INCENP
Aurora B
Borealin
HBXIP
Survivin HBXIP
Pro-Caspase 9
APOPTOSIS
Survivin
Survivin XIAP
Caspase 9
Caspase 7
Caspase 3
SMAC
XIAP
Hsp90
Survivin
Survivin Hsp90
SMAC
Survivin

Figure 3.


Ang1
VEGF
IGF-1
EGF
GM-CSF
HER-2
Tie2
VEGFR
IGF-1R
EGFR
GM-CSFR
1,2
3-5
PI3K
6,7
COX2 (PTGS2)
15,16
Bcr-Abl
24
AKT
8-10
IKK complex
PP2A
GM-CSFR
GM-CSF
Ras
mTOR1
11
AKAP149
PKA
HER-2
1,2
Raf-1
p70S6K1
MDM2
EGFR
3-5
EGF
20-23
MEK1/2
TGFβ
Smad 2/3
TGFβR I/II
30
ERK 1/2
HIF-1a
FOXO1 FOXO3a
NF-κB
12-14
Hypoxia
p53
Smad 4
CBP
32
BIRC5
Rb E2F4
TCF-4
33
HIF-1a
β-Catenin
p38 MAPK
29
E2F 1/2/3
STAT3
Frizzled-LRP5/6
31
Wnt-2
APC
MKK 3/6
CDK 2/4
25,26
27,28
CDK1
NICD
PKA
Cellular Stress
Survivin
Hypoxia
Notch-1
Jagged 1
γ-secretase
34,35
JAK2
17-19
24
Bcr-Abl
IL-6R IL-11Rα gp130
GM-CSFR
IL-6 IL-11
GM-CSF
Direct stimulatory modification
Indirect stimulatory modification
Direct inhibitory modification
Ligand
Receptor
Kinase
Phosphatase
Transcription factor
Others
Proteasomal degradation