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DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
 APC | CDH1 | CTNBN1 | MYC | p53 | p63 | SLC2A1

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

OPINION

Survivin, cancer networks and pathway-directed drug discovery

Dario C. Altieri

Abstract | Although there is no shortage of potential targets for cancer therapeutics, we know of only a handful of molecules that are differentially expressed in cancer and intersect multiple pathways required for tumour maintenance. Survivin embodies these properties, and orchestrates integrated cellular networks that are essential for tumour cell proliferation and viability. Pursuing the nodal functions of survivin in cancer might lead to the development of global pathway inhibitors with unique therapeutic potential.

Our understanding of cancer genes has improved tremendously over the past three decades¹, but this has not translated into equivalent benefits to cancer patients. Cases of improved survival mostly reflect early detection or prevention, rather than improved treatment (*Surveillance Epidemiology and End Results*). The efficacy of mainstay cancer therapies, cytotoxics and radiation, has reached a plateau in the treatment of many cancers, and there is an urgent sense that improvements must now come from fresh approaches².

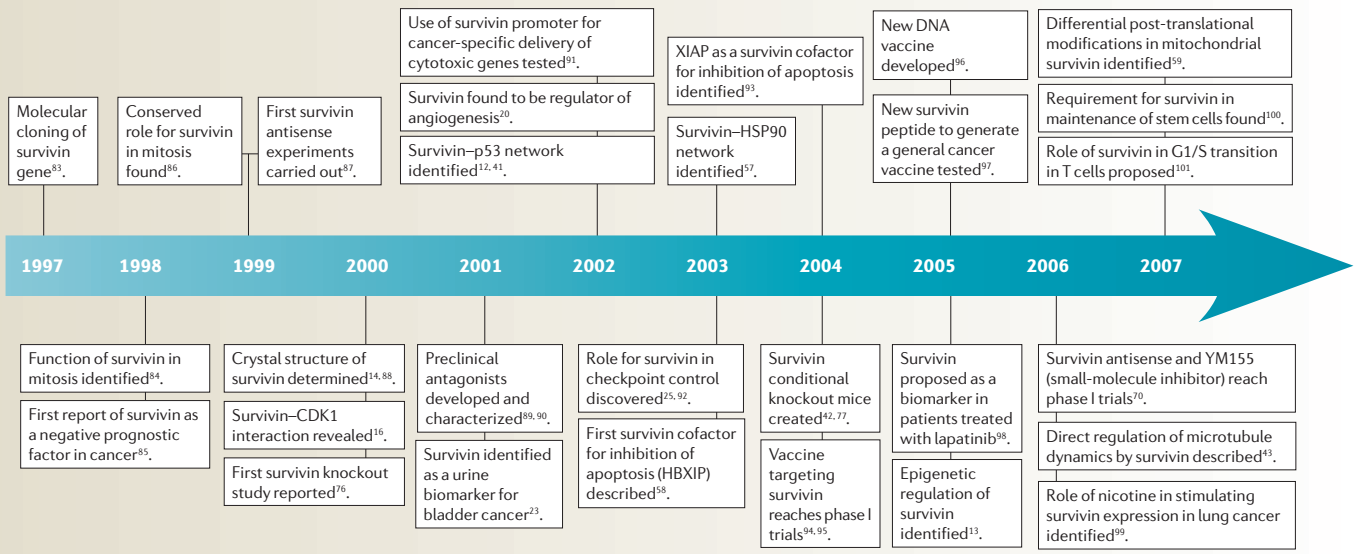
Backed by better knowledge of cancer genetics¹, we now attempt to produce drugs that eliminate tumour cells while sparing normal tissues³. This target-orientated approach is aimed specifically at genes whose products are involved in cancer, that are conceptually important for tumour maintenance, and that are 'druggable', typically by chemical-library screening or antibody production³. This strategy has produced a few impressive drugs that have revolutionized the treatment of certain tumours, for

instance, rituximab as therapy for non-Hodgkin lymphoma and imatinib in the management of chronic myelogenous leukaemia³. However, the overwhelming majority of cancers defy single-molecule-directed therapy, showing either transient benefits or no benefit at all. Even the rare tumours that are driven, at least at the onset, by only one signalling pathway become quickly resistant to single-molecule-directed therapy.

This probably reflects the extraordinary heterogeneity of cancer, which involves hundreds of mutated and deregulated genes, aberrant expression of microRNAs, genetic instability and aneuploidy¹. Leaving behind the fascination with 'magic bullet'-like drugs, new agents are being pursued for broader specificity in the hope that they will disable multiple signalling pathways⁴. What has been less appreciated, and is the focus of this article, is that investigating tumour diversity might lead to the identification of nodal proteins — proteins that are involved in multiple signalling mechanisms in tumour maintenance. Examples of nodal proteins that are upregulated, mutated or functionally exploited in cancer already exist. For instance, signalling through the epidermal growth factor receptor (EGFR) functions as a node, integrating extracellular cues with a panoply of downstream signalling responses, affecting cell proliferation, cell survival, differentiation and migration⁵. Similarly, heat shock protein 90 (HSP90, also known as HSP90AA1) is a node overseeing protein-folding quality control in all signalling hallmarks of cancer, such as cell proliferation, survival, immortalization, invasion, angiogenesis and resistance to growth-inhibitory signals⁶. Drugs targeting such nodal proteins might go beyond single-molecule antagonists and provide pathway inhibitors, globally affecting multiple signalling circuits in tumour cells, regardless of complexity, heterogeneity or genetic make-up.

Experimental evidence accumulated over the past 10 years suggests that survivin (encoded by *BIRC5*)⁷, a small inhibitor of apoptosis (IAP) protein⁸ sharply differentially expressed in cancer⁷, might be another paradigm of a nodal protein, with unique prospects for drug discovery. Good progress has been made in elucidating the function of survivin, and important milestones have been achieved in both basic and translational research (TIMELINE). This article will not deal with the specific aspects of survivin biology: several excellent reviews covering these topics have been published, and the reader is directed to these articles for a more in-depth perspective^{9–11}. Here, an effort will be made

Timeline | Milestones in survivin research



CDK1, cyclin-dependent kinase 1; HSP90, heat shock protein 90; XIAP, X-linked inhibitor of apoptosis protein.

to build a unifying model for the multiple functions of survivin, the implications of these functions for tumour maintenance and their suitability for novel cancer therapeutics.

The multiple facets of survivin

One unifying thread stands out in the biology of survivin: its link to multiple pathways of cellular homeostasis. To molecular biologists, survivin is encoded by a complex gene, which is extensively alternatively spliced, and its expression is finely regulated by transcriptional¹² and post-transcriptional mechanisms¹³. Biochemists view survivin as a structurally unique IAP protein¹⁴, expressed in several subcellular compartments¹⁵ and dynamically regulated by multiple post-translational mechanisms^{16,17}. To cell biologists, survivin is an essential regulator of cell division, a modulator of apoptotic and non-apoptotic cell death, and a stress response factor ensuring continued cell proliferation and cell survival in the face of unfavourable milieus^{7,9}. Survivin is a master switch of organ and tissue homeostasis in the eyes of geneticists, required to preserve the viability and proliferative potential of multiple tissue districts¹⁸. To cancer biologists, survivin is one of the most tumour-specific molecules¹⁹, which antagonizes apoptosis⁷, promotes tumour-associated angiogenesis²⁰ and acts as a resistance factor to various anticancer therapies^{20,21}. Finally, to clinical investigators, survivin is a model for bench-to-bedside cancer research, a molecular signature of unfavourable disease outcome²²,

a diagnostic biomarker of tumour onset and recurrence²³, and a validated target for cancer drug discovery²⁴. Although these are disparate and seemingly distant fields of investigation, only a holistic understanding of survivin function across the different areas can unlock the potential of the survivin networks for novel cancer therapeutics.

Unifying the survivin controversies

Although the published record on survivin is fairly consistent across areas of investigation, dissecting the multifaceted complexity of its biology (BOX 1) has not been without controversies. These largely reflected gaps in our knowledge and, with a better understanding of the pathways involved, most of the debated issues found reasonable explanations. One hot topic revolved around the function, or functions, of survivin during cell division. In particular, it was difficult to reconcile a somewhat controversial localization of survivin to microtubules²⁵ and its proposed function in spindle formation⁷ with the role of survivin as a kinetochore-associated chromosomal passenger protein, a group of molecules known to regulate late-phase mitosis or cytokinesis⁹. New data have probably settled the debate. It is now accepted that survivin exists in immunochemically distinct pools localized in various subcellular compartments, including kinetochores and microtubules¹⁵, and that chromosomal passenger proteins, including survivin²⁶, do contribute to spindle assembly²⁷ by nucleating microtubules around mitotic chromosomes.

It was also extensively debated whether survivin was, in fact, a genuine inhibitor of apoptosis. Despite unanimous published evidence that survivin antagonized various forms of cell death *in vivo*, there was no functional or structural data that it did so by inhibiting caspases¹⁴, as was expected from an IAP⁸. Moreover, a survivin orthologue in *Caenorhabditis elegans*, a model organism extensively used to study cell death, did not inhibit apoptosis²⁸. With new data becoming available, this debate is also probably settled. We now know that except for one member, X-linked IAP (XIAP, also known as *BIRC4*), all IAPs antagonize apoptosis independently of direct caspase inhibition⁸, that survivin-like molecules in model organisms, such as *Drosophila melanogaster*²⁹ or yeast³⁰, do in fact inhibit apoptosis, and that survivin preferentially antagonizes cell death upstream of effector caspases³¹, a pathway that is not operative in *C. elegans*.

Survivin nodes

One of the signature features of survivin is the surprisingly high number of molecules, regulators, transcriptional networks and modifiers that, directly or indirectly, are involved in its functions. Such complexity cannot be appreciated by thinking of survivin in isolation, but by delineating connectivity maps that link survivin to multiple signalling circuits³². Such a systems-biology approach has been used to recapitulate the extraordinary heterogeneity of tumour cells, particularly with respect to multilayered

organization, modularity into semi-autonomous subsystems and redundancy⁴. Using this approach as a working model, survivin emerges as a central node in multiple cellular networks, from which parallel signalling pathways branch out to regulate additional aspects in cellular homeostasis.

The survivin cell-division network

Inspecting the panoply of interactions that characterize the role of survivin in cell division offers a glimpse into the complexity and diversity of the survivin network (FIG. 1).

Subnetwork 1: survivin functions in the chromosomal passenger complex. As indicated above, survivin is a chromosomal passenger protein³³, and targets other molecules in a chromosomal passenger complex, including Aurora kinase B (*AURKB*), inner centromere protein antigens (*INCENP*) and borealin (also known as *CDCA8*), to kinetochores³⁴.

This trafficking pathway is essential, and mislocalization of the complex causes catastrophic mitotic defects. It is not surprising that parallel mechanisms have evolved to ensure redundancy and independently contribute to kinetochore targeting of the chromosomal passenger complex. What is surprising is that these additional pathways are also centred on survivin. In yeast, these include binding of survivin to a regulator of chromosome segregation called shugoshin 2 (REF. 35), and recruitment of the mitotic exit network³⁶, which contributes to inactivation of cyclin-dependent kinases (CDKs), completion of cytokinesis and initiation of G1 gene expression. In human cells regulatory phosphorylation of survivin by Aurora kinase B³⁷ and sequential cycles of survivin ubiquitylation and deubiquitylation by the enzyme hFAM¹⁷ have been described. Although these are intriguing similarities, it is also likely that survivin orthologues in different model organisms have evolved considerable diversity in molecular interactions and cellular functions. A parallel trafficking subsystem has emerged that implicates the RAN-GTP pathway, by binding of survivin to exportin 1 (*XPO1*, the human homologue of yeast *Crm1*) (REF. 11), which is a RAN effector molecule that regulates kinetochore fibre assembly, or through the recruitment of the RCC1 family protein TD60 (also known as *RCC2*) (REF. 38), which is a guanine nucleotide-exchange factor that also assembles in the chromosomal passenger complex (FIG. 1).

Box 1 | The main features of survivin

Biochemistry

- Member of the inhibitor of apoptosis (IAP) family, containing a single baculovirus IAP repeat (BIR) (see figure).
- Homodimeric structure.
- Known phosphorylation sites (kinase): Thr34 (cyclin-dependent kinase 1 (CDK1)); Thr117 (Aurora kinase B); Ser20 (protein kinase A (PKA)).
- Other known post-translational modifications: ubiquitylation of Lys23, Lys62, Lys78 and Lys79 by ubiquitin Lys63 ligases.
- Known binding sites for protein partners validated by direct protein–protein interactions, NMR, X-ray crystallography and site-directed mutagenesis: polymerized microtubules and electrostatic interactions with chromosomal passenger proteins, (carboxy terminus α helices, inner centromere protein antigens (*INCENP*), Lys112 and Lys120; borealin, Lys110, Lys121 and Arg132); *XPO1* /nuclear export sequence (NES, Val89–Leu98); *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- Δ Ex-3 C terminus); borealin and *INCENP* (dimer interface hydrophobic pocket: Leu6, Trp10, Phe93, Phe101 and Leu102).

Cell biology

- Cell-cycle expression at mitosis (deregulated in cancer with high expression in interphase).
- Localization to the mitotic apparatus: centrosomes, kinetochores, mitotic spindle microtubules, spindle poles, central spindle midzone and midbodies.
- Other known subcellular localizations: cytosol, mitochondria and nuclei.

Functions

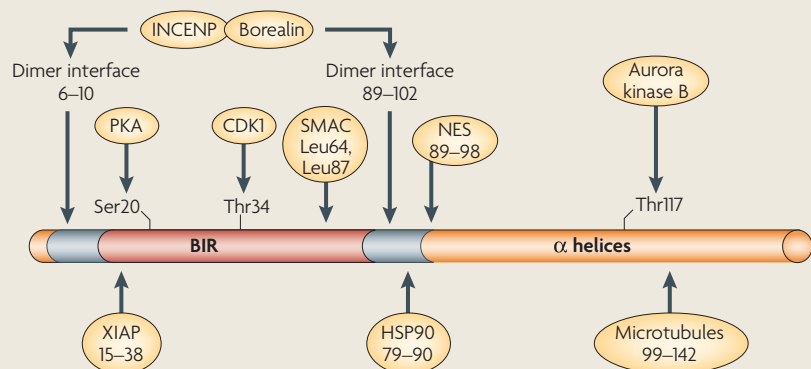
- Evolutionarily conserved, essential role in mitosis.
- Role in chromosomal attachment, spindle-assembly checkpoint.
- Role in S-phase progression (thymocytes, activated T cells).
- Inhibition of caspase-dependent apoptosis and caspase-independent cell death.
- Inhibition of mitochondrial and death receptor (tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis.

Tissue expression

- Ubiquitous in embryonic and fetal development.
- Undetectable in most adult tissues.
- Low expression in adult basal colonic epithelium and CD34⁺ haematopoietic progenitors.
- Overexpressed in all human cancers, independently of mitotic index.

Phenotypes of knockout studies in mice

- Germline knockout: embryonic lethality (E 3.5)⁷⁶.
- Conditional knockout in thymocytes: impaired cell proliferation⁷⁷, cell cycle arrest, mitotic spindle defects and apoptosis⁷².
- Conditional knockout in neuronal precursors⁷⁸: perinatal lethality, reduction in brain size, apoptosis and increased caspase 3 and 9 activity.
- Conditional knockout in endothelial cells⁷⁹: embryonic lethality, haemorrhages, neural tube closure defects and hypoplastic endocardial cushions.
- Conditional knockout in haematopoietic progenitors⁸⁰: mortality, bone-marrow ablation, loss of haematopoietic progenitors and erythropoiesis defects.



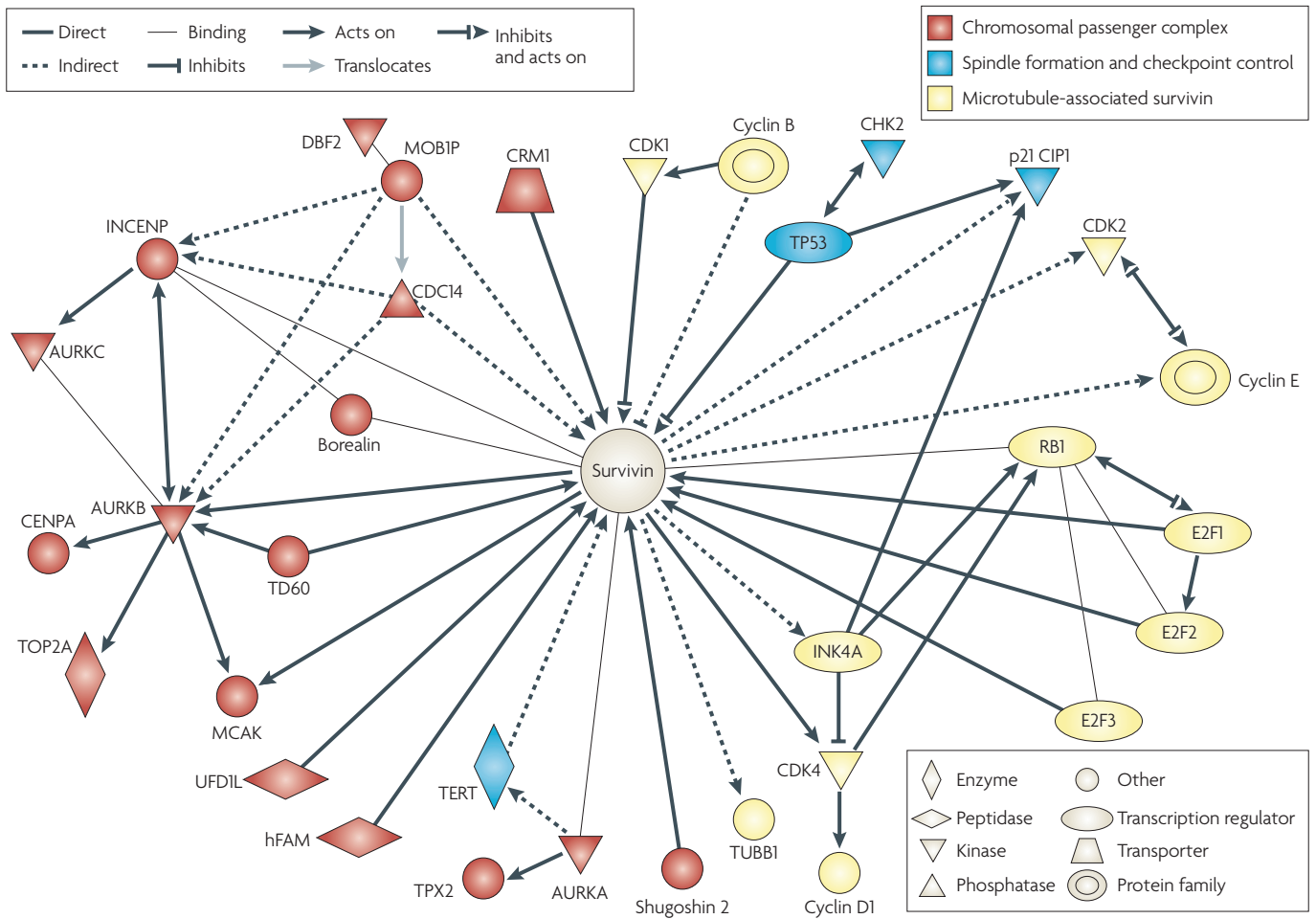


Figure 1 | Connectivity map of the survivin networks at cell division. Each of the node shapes denotes the function of the interacting protein or group of proteins. The connectivity map was generated from available published data using the Ingenuity Pathway Analysis (Ingenuity Systems). Data are compiled from interactions validated in multiple model organisms. Not all proteins indicated have human homologues. AURK, Aurora kinase;

CDC, cell-division cycle; CDK, cyclin-dependent kinase; CENPA, centromere protein A; CHK2, checkpoint kinase 2; CRM1, chromosome region maintenance protein 1; INCENP, inner centromere protein antigens; MCAK, mitotic centromere-associated kinesin; RB1, retinoblastoma 1; TERT, telomerase reverse-transcriptase; TOP2A, topoisomerase II α ; TUBB1, tubulin β 1; UFD, ubiquitin fusion degradation.

Subnetwork 2: spindle formation and checkpoint control. Once localized in the chromosomal passenger complex, survivin contributes to chromatin-associated spindle formation²⁷. This process involves the stimulation of Aurora kinase B activity, which in turn phosphorylates the mitotic centromere-associated kinesin (MCAK, also known as *KIF2C*)²⁶ and removes its microtubule depolymerizing activity. The role of survivin in spindle assembly connects to parallel pathways of genomic fidelity, in which survivin has been characterized as a sensor of kinetochore–microtubule attachment³⁹, a component of the spindle-assembly checkpoint that is activated by a lack of microtubule tension²⁵. It is from this checkpoint function of genomic surveillance that the survivin network further intersects with mechanisms of apoptosis regulation.

Accordingly, activation of the checkpoint kinase CHK2 (encoded by *CHEK2*) by DNA damage stimulates a rapid discharge of the mitochondrial pool of survivin in the cytosol⁴⁰. This pathway does not seem to participate in cell division, but preserves the viability of tumour cells during a protracted G2 block by antagonizing DNA-damage-induced apoptosis⁴⁰. A parallel survivin–p53 subsystem has evolved to oppose this effect. DNA damage also stabilizes p53, but in this case p53 functions as an efficient repressor of *BIRC5* transcription, through occupancy of a binding site in the *BIRC5* promoter⁴¹, changes in chromatin structure affecting promoter accessibility¹², or epigenetic modifications involving DNA cytosine methyltransferase 1 (REF. 13). The net effect is an abrupt lowering of survivin levels, which itself

is a stimulus to stabilize p53 (REF. 42), and further reduce survivin expression. Therefore, balancing a survival effect of CHK2 (REF. 40), the p53 subsystem aims to eliminate survivin expression during DNA damage, thus tilting the balance towards stable cell-cycle arrest and apoptosis. One can speculate that loss of p53, as occurs frequently in human cancer, might cause unrestrained survivin expression resulting in enhanced cell viability, impaired checkpoint function and increased propensity to aneuploidy.

Subnetwork 3: microtubule-associated survivin. In addition to its localization in the chromosomal passenger complex, a fraction of mitotic survivin directly assembles on polymerized microtubules⁷. Similarly to kinetochore survivin²⁶, microtubule-associated

survivin contributes to spindle formation, but this involves a different pathway of enhanced microtubule stability through suppression of microtubule dynamics, reduction of microtubule nucleation from centrosomes, increased acetylated tubulin content and increased resistance to nocodazole-induced microtubule depolymerization, in a pathway that is independent of Aurora kinase B⁴³. This survivin network also branches out to parallel pathways of genomic integrity and intersects mechanisms of apoptosis. Through its association with CDK1 (also known as *CDC2*), microtubule-bound survivin becomes phosphorylated at mitosis on Thr34 (REF. 16). This step is crucial to stabilize survivin at mitosis and efficiently counteract apoptosis of dividing cells, or in response to spindle poisons¹⁶. The anti-apoptotic environment created by CDK1 is not limited to its effects on survivin. CDK1 phosphorylation of *caspase 9*, an upstream initiator of mitochondrial cell death, abolishes its anti-apoptotic activity and antagonizes cell death induced by anti-mitotic agents⁴⁴. Conversely, pharmacological inhibition of CDK1 removes this cytoprotective environment, and triggers tumour cell death, either in sequential combination with taxanes⁴⁵, or selectively in tumours driven by the *MYC* oncogene⁴⁶ (FIG. 1).

The survivin anti-apoptotic network

The role of survivin in the inhibition of apoptosis has a similar degree of complexity, connecting to multiple parallel pathways that regulate gene expression, protein–protein interactions and mitochondrial functions (FIG. 2).

Subnetwork 1: providing a heightened cell-survival threshold. In addition to a stable and protracted mitotic arrest, acute lowering of survivin expression (for instance, using antisense, small interfering RNA, ribozymes or dominant-negative mutants) has often been associated with spontaneous apoptosis, depending on the cell type and its complement of checkpoints. Accordingly, pathways that regulate gene expression and control protein stability extensively intersect with the survivin cytoprotection network. Many prototype tumour-suppressor genes result in efficient silencing of the *BIRC5* promoter. These include the adenomatous polyposis coli protein⁴⁷, which is often deleted or mutated in colorectal cancer, p53 (see above), fragile histidine triad gene (*FHIT*)⁴⁸, which is a pro-apoptotic molecule that binds and hydrolyses diadenosine polyphosphates, and *PML4*, a pro-apoptotic promyelocytic

leukaemia protein⁴⁹. By contrast, oncogenic factors have been shown to promote *BIRC5* transcription. This is the case for *TCF4*–*β-catenin*⁵⁰, a developmentally regulated transcriptional activator complex participating in colon cancer, signal transduction and activator of transcription 3 (*STAT3*)⁵¹, which is an oncogenic transcription factor involved in cytokine signalling, and a group of E2F transcription factors⁵², which function in the G1/S transition of the cell cycle. Of these regulators, discrete binding sites on the *BIRC5* promoter have been identified for *TCF4*, p53 and *STAT3* (REF. 53), suggesting that these molecules might directly control *BIRC5* expression. A second post-transcriptional network that controls survivin mRNA or protein stability has also been characterized. This involves several factors: the mammalian target of rapamycin (mTOR, also known as *FRAP1*), which is required for stability and translation of a cytosolic pool of *BIRC5* mRNA⁵⁴; intermediaries of growth factor receptor signalling, especially the phosphatidylinositol 3-kinase–Akt axis, which has been frequently implicated in the modulation of survivin levels⁵⁵; CDK1 phosphorylation, which promotes increased survivin stability at mitosis¹⁶; and binding of survivin to molecular chaperones, including the aryl hydrocarbon receptor-interacting protein (*AIP*)⁵⁶, and HSP90 (REF. 57), which participate in survivin stability and subcellular trafficking pathways. Pharmacological antagonists of some of these pathways are being tested for cancer therapy, and their ability to lower survivin levels may contribute to their anticancer activity. In addition, changes in survivin expression could provide an accessible biomarker of target validation for patients treated with inhibitors of HSP90 (17-AAG), the EGFR family (lapatinib) or CDK1 (flavopiridol).

Subnetwork 2: intermolecular cooperation.

One of the crucial features of this cytoprotective network is that it relies on physical interactions between survivin and other adaptor or cofactor molecules. This may explain why earlier studies with isolated recombinant survivin in a cell-free system did not show anti-apoptotic effects¹⁴. In the cytosol, survivin associates with the hepatitis B X-interacting protein (*HBXIP*), and this complex, but not either protein alone, binds caspase 9 and inhibits mitochondrial apoptosis⁵⁸. Survivin exhibits parallel interactions with other members of the IAP gene family. One interaction involves XIAP, which binds the pool of survivin released from mitochondria in response

to cell-death stimuli⁵⁹, resulting in increased XIAP stability against proteasomal degradation and inhibition of apoptosis *in vivo*³¹. This pathway can be recapitulated *in vitro* with recombinant proteins with synergistic inhibition of caspase 3 and 9 activity. Assembly of the survivin–XIAP complex *in vivo* is regulated in subcellular compartments, and phosphorylation of survivin on Ser20 by protein kinase A in the cytosol, but not in mitochondria, disassembles the complex, and abolishes its anti-apoptotic function⁵⁹. The interaction between survivin and the RAN–GTP effector XPO1 may also bridge cell division and cytoprotective networks, as it may be required to localize survivin for apoptosis inhibition in the cytosol. Conversely, a complex of survivin and cIAP1 (also known as *BIRC2*) might not participate in apoptosis inhibition, but seems to feed back on the regulation of survivin during cell division⁶⁰. Cells overexpressing cIAP1 displayed extensive mitotic defects, cytokinesis failure and a propensity for chromosomal instability, suggesting that a survivin–cIAP1 complex might antagonize the function of survivin in late-stage cell division⁶⁰. Finally, survivin has been implicated in heterodimeric interaction with at least some of its alternatively spliced forms⁶¹. With the caveats that these results were obtained using overexpression approaches, and that the balance of survivin dimers versus monomers *in vivo* is far from understood, it has been suggested that these complexes may also participate in cytoprotection⁶¹.

Subnetwork 3: mitochondrial dynamics.

Recent evidence suggests that survivin cytoprotection hinges on a pool of the molecule compartmentalized in mitochondria, and released in the cytosol in response to cell death stimuli³¹. Accordingly, there are multiple signalling pathways of mitochondrial homeostasis that connect to survivin cytoprotection. First, although it is as yet unclear how survivin is transported to mitochondria, its regulated association with molecular chaperones, *AIP*⁵⁶ or HSP90 (REF. 57), might contribute to this process, potentially through the import receptor complexes at the outer mitochondrial membrane, TOM20 (also known as *TOMM20*) and TOM70 (also known as *TOMM70A*). Consistent with this model, recent data from our laboratory have demonstrated using a cell-free import assay that survivin is actively imported in purified mitochondria (B. H. Kang and D.C.A., unpublished observations). Second, mitochondrial survivin is post-translationally modified, and this step is required for its anti-apoptotic

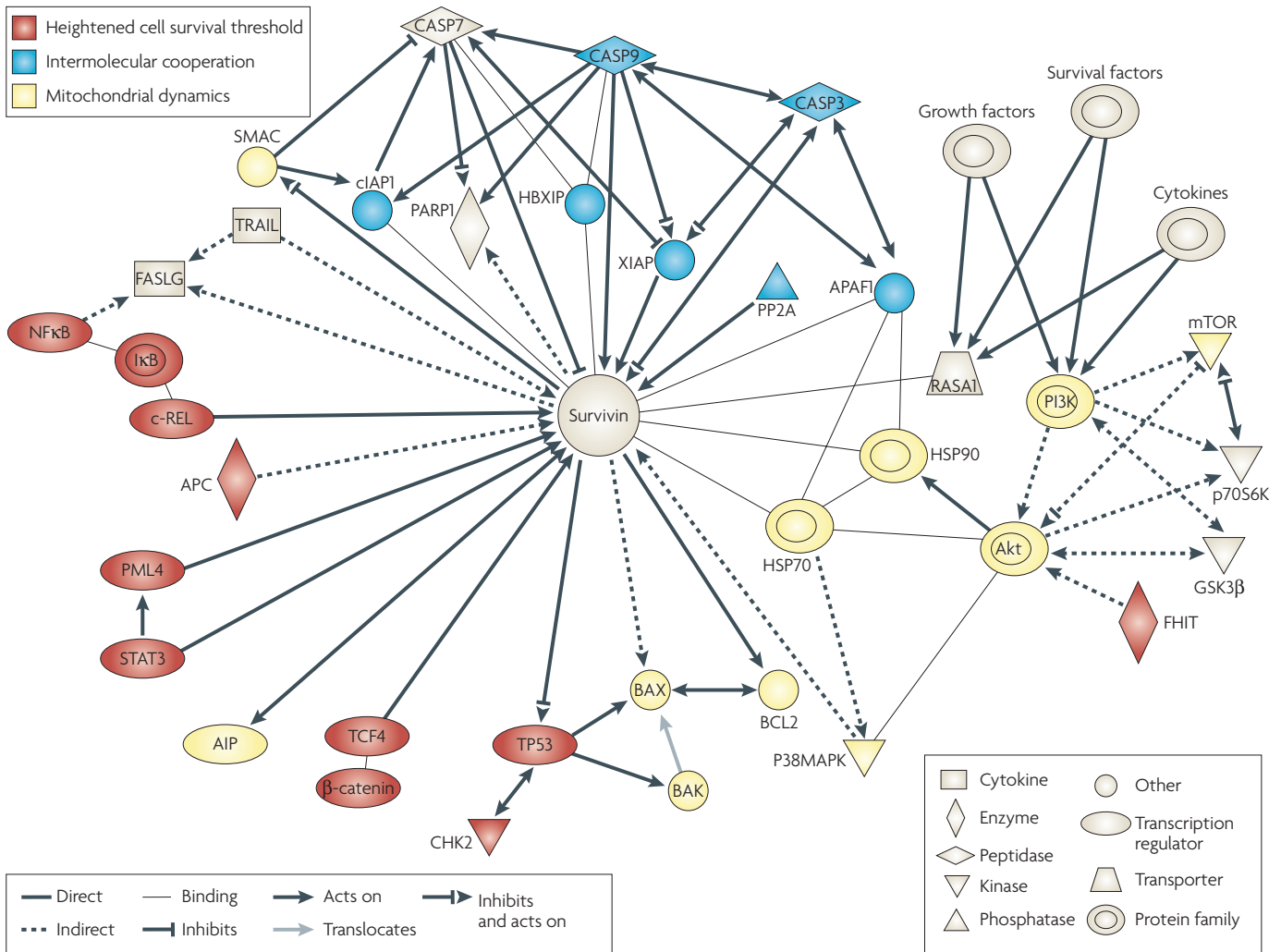


Figure 2 | Connectivity map of the survivin networks at cell death. The conditions to generate the connectivity map in the survivin cell death network are as described in Figure 1. Data are compiled from interactions validated in multiple model organisms. Not all proteins indicated have human homologues. AIP, aryl hydrocarbon receptor interacting protein; APAF1, apoptotic protease activating factor 1; APC, adenomatous polyposis coli; BAK, BCL2-antagonist/killer; BAX, BCL2-associated X protein; CASP, caspase; DIABLO (SMAC), direct inhibitor of apoptosis binding protein with low pH; GSK3β, glycogen synthase kinase 3β; FASLG, Fas

ligand; FHIT, fragile histidine triad; mTOR, mammalian target of rapamycin; HBXIP, hepatitis B X-interacting protein; HSP, heat shock protein; IAP, inhibitor of apoptosis protein; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor κB; PARP, poly(ADP-ribose) polymerase; PI3K, phosphatidylinositol 3-kinase; PML, promyelocytic leukemia; PP2A, protein phosphatase 2A; RASA, RAS p21 protein activator; STAT, signal transducer and activator of transcription; TCF4, transcription factor 4; TRAIL, tumour necrosis factor-related apoptosis inducing ligand; XIAP, X-linked inhibitor of apoptosis protein.

function. Only survivin that is kept unphosphorylated on Ser20 retains the ability to bind XIAP and antagonize cell death (see below), and this process may involve compartmentalized proximity in mitochondria between survivin and the broad spectrum phosphatase, PP2A, which dephosphorylates survivin on Ser20 (REF. 59). Third, once transported in mitochondria and properly processed, survivin binds SMAC (also known as *DIABLO*)⁶², a molecule that relieves the inhibitory effect of XIAP on caspases and thus promotes cell death. The actual physiological relevance of a survivin–SMAC complex *in vivo* has not been

fully established, a caveat that might apply to other reported interactions involving survivin, for instance, when using supra-physiological overexpression approaches. However, there have been reports that this interaction may regulate apoptosis directly, by sequestering SMAC away from XIAP⁶³, or indirectly, by preventing altogether its release from mitochondria⁶⁴.

In a parallel pathway, an alternatively spliced survivin variant, called survivin-ΔEx-3, containing a novel carboxy terminus sequence due to a frameshift, has been shown to localize to mitochondria, where it interacts with *BCL2* and inhibits caspase 3

activity⁶⁵. Because anti-apoptotic *BCL2* proteins function as inhibitors of mitochondrial permeability transition, this recognition would position survivin, or at least one of its spliced variants, in the regulation of mitochondrial membrane integrity. Variations of this pathway have been suggested, involving hyperphosphorylation of *BCL2*, and reduced activation of pro-apoptotic BCL2-associated X protein (*BAX*) by the survivin–Aurora kinase B complex, potentially upstream of caspase activation⁶⁶, thus further dampening mitochondrial permeability. It is too soon to tell whether a broader basis exists for a role of survivin

in mitochondrial homeostasis, but it is intriguing that survivin- Δ Ex-3 was recently shown to maintain mitochondrial membrane potential and control the production of reactive oxygen species in response to cell-death stimuli⁶⁷. It should be kept in mind that the actual abundance of survivin splice variants in tumour cells appears to be quite low. Although this does not negate *a priori* a role of these molecules in survivin regulation, definitive validation of this model awaits the availability of specific reagents capable of faithfully discriminating the various endogenous survivin isoforms in different cells and tissues.

Therefore, the survivin networks in cell division (FIG. 1) and cell death (FIG. 2) emerge as highly flexible signalling hubs, connecting to multiple independent pathways of cellular homeostasis (FIG. 3). It seems plausible to hypothesize that the cytoprotective and mitotic functions of survivin intersect at cell division. There is compelling experimental evidence to support this model, as interference with survivin expression and/or function in synchronized cultures often culminates in a form of apoptosis of dividing cells called mitotic catastrophe. However, this is probably not the whole story, and survivin cytoprotection is probably operative in interphase as well. This is consistent with the fact that tumour cells have constitutively high levels of survivin in interphase *in vivo* and that cell-cycle-regulated transcription of *BIRC5* at mitosis cannot account for the expression of endogenous survivin in transgenic mice⁶⁸, and with the dynamics of mitochondrial survivin, which are uncoupled from cell-cycle progression⁵⁹.

Survivin networks and drug discovery *A rationale for targeting survivin.*

Molecular profiling studies and retrospective analyses of patient cohorts have consistently identified the increased expression of survivin as a risk factor for cancer progression and poor prognosis⁶⁹. In breast cancer, survivin expression might also have a role in predicting recurrence²². Although it is possible that distinct subcellular pools of survivin might differentially influence prognosis¹¹, the survivin networks seem to confer on tumour cells a greater adaptability, proliferative capacity and resistance to cell death, which translates into a clinically worse disease. However, the nodal functions of survivin might constitute a unique Achilles' heel for cancer cells, as a non-redundant network of tumour maintenance that is unable to be circumvented (FIG. 3).

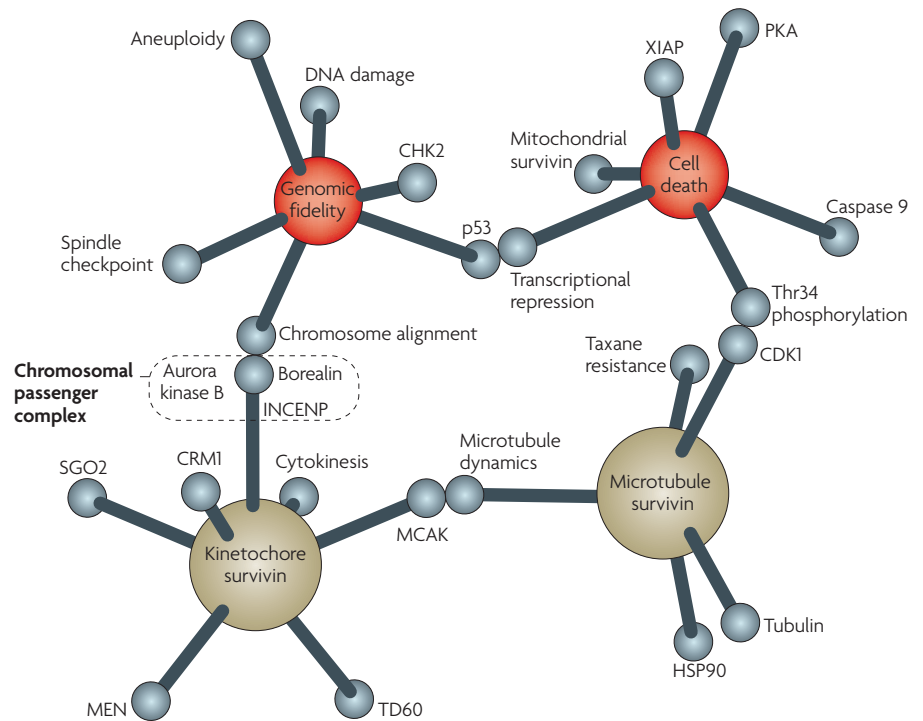


Figure 3 | Connectivity links between the survivin cell division and cell death networks. The functions of survivin intersect with mechanisms of cell division control, genomic fidelity, mitotic spindle assembly, subcellular trafficking, checkpoint regulation and apoptosis. CDK1, cyclin-dependent kinase 1; CRM1, chromosome region maintenance protein 1; HSP90, heat shock protein 90; INCENP, inner centromere protein antigens; MCAK, mitotic centromere-associated kinesin; MEN, mitotic exit network; PKA, protein kinase A; SGO2, shugoshin 2; XIAP, X-linked inhibitor of apoptosis protein.

Following this logic, putative survivin antagonists might function not as single-protein inhibitors³ but, in fact, as pathway inhibitors⁴ that are suitable for disabling multiple signalling circuits in tumours, regardless of their heterogeneity or genetic make-up. Although it is likely that some of the survivin networks become operative in a specific spatial-temporal context, for instance, as cells approach mitosis, or during the cellular stress response, therapeutic disabling of survivin may have global effects on tumour cells, conceptually similar to the therapeutic targeting of other nodal proteins in tumorigenesis — for instance, EGFR⁵ or HSP90 (REF. 6).

The portfolio of survivin antagonists.

Unfortunately, despite the efforts of many laboratories to elucidate the biology of survivin (TIMELINE), the portfolio of survivin antagonists available for clinical testing is small (TABLE 1). It includes molecules that specifically target survivin, including an antisense molecule (LY218130B) and transcriptional repressors (YM155 and EM-1421), but also compounds that appear to perturb survivin expression and/or function

(TABLE 1) indirectly, as part of a more global inhibition of cellular signalling pathways. Although potentially still valuable for anticancer activity, this makes it difficult to ascertain the relevance of each different signalling pathway being simultaneously targeted. The lack of a larger portfolio of truly survivin-directed antagonists probably reflects a common view in drug discovery that molecules that are not present on the cell surface, or that lack inhibitable catalytic activity, are not considered good targets⁴. In the case of survivin, one also has to acknowledge that crystallographic data reveal little in the way of potential drugable sites, which are typically structural pockets of suitable geometry and hydrophobicity. Despite these challenges, the survivin antagonists that have succeeded in reaching the clinic generated promising results. Of the two small-molecule inhibitors of *BIRC5* transcription, YM155 produced impressive clinical responses in phase I trials of heavily pretreated cancer patients⁷⁰, and EM-1421 generated encouraging results as a topical application in cervical intraepithelial neoplasia and is now in phase I trials⁷¹. Several phase II trials were also announced for the survivin antisense

Table 1 | Portfolio of survivin antagonists and status of their clinical development

| Therapeutic approach | Compounds | Preclinical trials | Clinical development* |
|--|---|--------------------|---|
| Antisense | LY2181308 | Completed | Phase I trial completed Phase II trial announced |
| Molecular antagonists | Ribozyme RNA interference | Ongoing | Not started |
| Gene therapy | Dominant interfering mutants (C84A; T34A) | Ongoing | Not started |
| | <i>BIRC5</i> promoter for tumour-specific transcription of cytotoxic gene(s) [‡] | Ongoing | Planned |
| Transcriptional repressors | EM-1421 (tetra- <i>O</i> -methyl nordihydroguaiaretic acid) | Completed | Phase I trial ongoing |
| | YM155 | Completed | Phase I trials completed Phase II trials ongoing |
| Small molecule antagonists of other pathways | STAT3 (STA-21; WP1066) | Completed | Phase I trial planned |
| | CDK1 (flavopiridol) | Completed | Phase II trial ongoing (NCT00098371) |
| | TCF (SDX-308) | Completed | Phase II trial ongoing |
| | HSP90 (17-AAG) | Completed | Phase I and II trials ongoing (NCT00096005, NCT00117988, NCT00096109) |
| | ERBB2 (lapatinib or Tykerb) | Completed | Phase III trials ongoing (NCT00374322) |
| Immunotherapy | Autologous CTL pulsed with survivin-primed dendritic cells | Completed | Phase I and II trials ongoing |
| | Oral DNA vaccine (survivin peptide) | Ongoing | Planned |
| Peptidomimetic | Combined survivin and HSP90 antagonist (shepherdin) | Ongoing | Ongoing |

*Numbers in parentheses are identifiers on ClinicalTrials.gov. [‡]Because of its tumour-specific transcription, the *BIRC5* promoter has been used to drive expression of cytotoxic 'payload' genes selectively in tumour cells⁸¹. *BIRC5* (survivin), baculoviral inhibitor of apoptosis protein repeat-containing protein 5; CDK1, cyclin-dependent kinase 1; CTL, cytotoxic T lymphocyte; HSP90, heat shock protein 90; STAT3, signal transducer and activator of transcription 3; TCF, T-cell factor. Modified, with permission, from REF 82 (2006) © American Association for Cancer Research.

molecule LY2181308 (ClinicalTrials.gov), and for cancer vaccination protocols using survivin peptides⁷². However, this relatively small assortment of agents (TABLE 1) might not fully unlock the potential of survivin as a nodal cancer drug target.

Novel approaches to targeting survivin.

Thinking of the survivin networks as outlined above might offer fresh opportunities for drug discovery. Although traditionally not viewed as easy to target, there is ample precedent that disruption of protein–protein interactions, especially those involving apoptosis regulators, creates meaningful anticancer activity, manageable toxicity and drug-like properties that warrant clinical testing²⁴. In this context, proof-of-principle agents that disrupt the physical complex between survivin and other network components — for instance, HSP90 — have been identified⁷³. A prototype of these compounds, the peptidomimetic shepherdin, which is a combined survivin and HSP90 inhibitor, exhibited strong anticancer activity *in vivo* with no toxicity for normal tissues⁷³, and its clinical development is now underway. It is conceivable that molecular disruption of other survivin-containing protein complexes, for instance, those involving SMAC⁶² or XIAP⁵⁹, might disable survivin cytoprotection and trigger tumour cell death alone or, more

likely, in combination with conventional⁴⁵ or targeted⁴⁶ anticancer agents, given the extensive molecular and genetic complexity of most tumours by the time they are discovered.

Second, experimental evidence suggests that the survivin networks are finely regulated, and even relatively small changes in survivin post-translational modifications^{37,45} or binding to protein partners^{57,59} can cause protein mislocalization, disruption of molecular interactions and accelerated proteasomal destruction. On the basis of available evidence, it seems tumour cells cannot recover from loss or deregulation of survivin, and undergo immediate cell-cycle arrest and spontaneous cell death. Therefore, it is possible that the high-affinity binding of a small molecule to survivin might deregulate node dynamics and trigger cell-cycle arrest and apoptosis. High-throughput, affinity-based screening for small molecules that interact with apoptosis regulators is feasible, and candidate drug-like compounds with these characteristics, for instance, the BH3 mimetic ABT-737, are already in the clinic²⁴.

Survivin antagonists and cancer networks

One intriguing feature of the survivin networks is that many of the survivin-binding partners themselves behave as oncoproteins, as they are overexpressed, mutated or

functionally exploited in tumours, as opposed to normal tissues ([Supplementary information S1](#) (figure)).

Differences in survivin networks in tumour cells?

To explain this preponderance of oncoproteins that are functionally associated with survivin, one can speculate that the survivin networks might be qualitatively different in cancer, namely that they might rely on a host of protein partners that are selectively or differentially used by tumour cells. However, this has yet to be demonstrated experimentally. This idea may help reconcile some unexpected findings regarding the use of survivin antagonists as anticancer agents, and their potential risk for toxicity in humans. There is a unanimous consensus that survivin is essential during development, and might also have a crucial homeostatic function in certain adult tissues^{10,18}. Despite this, survivin antagonists, at least those tested so far, were generally well tolerated in clinical and preclinical studies, with modest side effects potentially unrelated to drug treatment. For instance, in the case of two YM155 phase I trials, the most common adverse events observed included pyrexia, arthralgia, nausea, fatigue and mucosal inflammation in one patient series (41 patients (31 male and 10 female), median age 61 years)⁷⁰, and fatigue,

microalbuminaemia, pyrexia and anaemia in a second patient series (34 patients (24 male and 10 female), median age 60 years)⁷⁴. The results of the LY2181308 phase I trial are not published, but the fact that this regimen has now been moved to phase II studies suggests that potential toxicities were manageable. Clearly, given the multiplicity of functions of survivin, and a few reports suggesting its expression in normal, differentiated tissues, it remains possible that survivin-based therapies, especially when new classes of antagonists become available, might cause as yet unseen side effects in humans. However, an alternative possibility can be formulated: targeting survivin might selectively affect the qualitatively different networks organized in tumour cells (Supplementary information S1 (figure)), but leave survivin functions unscathed in normal tissues.

Although this hypothesis awaits confirmation from a more in-depth understanding of the multiple facets of survivin networks in normal and tumour cells, the idea that signalling pathways might be preferentially, or even exclusively, operational in cancer is not without precedent, and is reminiscent of the concept of 'oncogene addiction', in which tumours become dependent on crucial oncoprotein(s) for their maintenance⁷⁵. It is not known to what extent this occurs *in vivo*⁷⁵, but it is clear that certain tumour characteristics confer sensitivity to molecularly targeted drugs — for example, EGFR antagonists⁵ — and this typically occurs with minimal side effects. In this context, it is possible that at least certain tumours might become 'addicted' to the survivin networks, offering not only therapeutic prospects for individualized treatment by survivin antagonists, but also a valuable therapeutic window to limit unwanted side effects. This may alleviate concerns that survivin-based therapeutics might produce unacceptable toxicity owing to global inhibition of cell proliferation, especially in pivotal cellular compartments, such as T cells and haematopoietic progenitors, in which survivin has been shown to play an important homeostatic role. Despite the fact that these considerations apply to any anti-mitotic agent, many of which are currently widely used to treat cancer, early clinical testing with the survivin suppressant YM155 did not uncover bone-marrow toxicity or a heightened incidence of infections^{70,74}.

Conclusions and perspectives

Ten years of studies have validated a pivotal role for survivin in tumour cell survival. Although the details of the multiple pathways

emanating from the survivin networks are yet to be fully elucidated, there is a consensus that survivin is an essential cancer gene and an appropriate target for drug discovery. The view presented here is that this is centred on the role of survivin as a nodal cancer protein, orchestrating extensive, and potentially tumour-specific, signalling networks.

Despite the fact that survivin is not a traditional drug target — that is, it is not an enzyme or a cell-surface molecule — its unique nodal properties imply that even relatively subtle perturbations of its expression, stability or binding to associated molecules could irreversibly impair tumour cell viability. This uniquely flexible approach for drug discovery, combined with the possibility for fewer side effects, might make survivin antagonists attractive global pathway inhibitors, ideally suited to overcome the extraordinary heterogeneity of human cancer.

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DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
 AURKB | BAX | BIRC2 | BIRC4 | BIRC5 | caspase_9 | CDC2 | CDCA8 | CHEK2 | CTNNB1 | DIABLO | EGFR | HSP90AA1 | INCENP | KIF2C | MYC | p53 | PML4 | STAT3 | TCF4 | TOMM20 | TOMM70A | XPO1 | β -catenin

FURTHER INFORMATION

Dario C. Altieri's homepage: <http://www.umassmed.edu/cancerbiology/index.aspx>
 Surveillance Epidemiology and End Results: <http://seer.cancer.gov>
 ClinicalTrials.gov: <http://www.clinicaltrials.gov/show/NCT00415155>
 Ingenuity Systems: <http://www.ingenuity.com>

SUPPLEMENTARY INFORMATION

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