Review

Regulation of Cancer Cell Survival by BCL2 Family Members upon Prolonged Mitotic Arrest: Opportunities for Anticancer Therapy

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Abstract. *Attacking cancer cell survival defense by targeting B-Cell Lymphoma 2 (BCL2) family of anti-apoptotic proteins may provide a powerful means to improve chemotherapy efficiency. This could be particularly relevant to anti-mitotic-based therapy, where tumor response relates to a competing network between mitotic cell death signaling and mitotic slippage as an adaptative response to a leaky mitotic checkpoint. In this review, we focus on recent findings that point out the major role played by BCL2 family members in response to anti-mitotic agents, which reveal dependence of cancer cell survival on BCL2 homologs during mitotic arrest and after mitotic slippage. Finally, we discuss pre-clinical data combining anti-mitotic agents with BCL2 inhibitors.*

Key apoptosis regulators contribute to the aberrant survival of cancer cells and to their resistance to chemotherapy. Their targeting thus appears to be an exciting therapeutic strategy for cancer, that could induce cell death or enhance chemotherapeutic efficiency if the exact molecular mechanisms at stake are understood. The apoptotic machinery is composed of the extrinsic pathway which receives and processes extracellular death signals, and the intrinsic (or mitochondrial) pathway, which senses and integrates a variety of signals of intracellular origin. Both pathways trigger a cascade of proteolysis that relies on caspases, whose activation culminates in cell disassembly.

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The intrinsic pathway is tightly regulated by members of the BCL2 family, which is composed of anti- and pro-apoptotic proteins. The multi-domain proteins such as BAX and BAK, which share 3 domains of homology with BCL2 (BH1-3 domains) and BH3-only proteins, such as BID, BIM, PUMA, or NOXA are pro-apoptotic proteins, whereas BCL2 homologs (BCL2, BCLXL, induced myeloid cell leukemia differentiation protein (MCL1), *etc*) promote survival. Mechanistically, BCL2 homologs prevent a subset of BH3-only proteins (BIM, BID, PUMA) from interacting with multi-domain proteins and from activating them [1], thereby preventing these proteins from triggering mitochondrial outer membrane permeabilization (MOMP) and the subsequent mitochondrial release of apoptogenic factors, such as cytochrome *c* or SMAC/Diablo (2). BCL2 homologs exert their anti-apoptotic function by engaging in an integrated network of interactions wherein a well-characterized hydrophobic groove at their surface binds to the BH3 domain of their proapoptotic counterparts. Notably, there are promiscuous but also selective interactions between BCL2 homologs and multi-domain or BH3-only proteins due to subtle, yet, significant differences in the BH3-binding interface of each BCL2 homolog. For instance, BIM or PUMA interact with all known BCL2 homologs whereas BAD interacts preferentially with BCL2 and BCLXL and NOXA with MCL1 (3). Alteration at any node of this so-called BCL2 network of interactions (by changes in the expression levels of BCL2 family members, by regulation of alternative splicing, by post-translational modifications, *etc*) is expected to impact on apoptotic sensitivity.

BCL2 homologs are frequently overexpressed in cancer cells, including solid tumors. They contribute to maintaining the survival of cancer cells in the face of constitutive death signals associated with oncogenic signaling [4], and to increasing their resistance against conventional chemotherapy. As discussed below, BCL2/BCLXL inhibitors

are now available in the therapeutic armamentarium in oncology; it is of major importance to define which therapeutic combination including such inhibitors could benefit cancer patients. Of particular interest is the combination of BCL2 inhibitors with anti-mitotic agents such as taxanes or vinca alkaloids. Indeed, the latter compounds are widely used in the treatment of solid tumors and in hematological cancer. They block mitosis progression and cell division by poisoning microtubules, and a great deal of their efficacy relies on their ability to trigger cell death during prolonged mitotic arrest. Even though microtubule inhibitors give good results in the clinic, innate or acquired resistance eventually occurs through various pathways. In addition to increased expression of drug transporter [Multidrug Resistance phenotype (MDR)] or target (tubulin) mutations, specific defects in triggering cell death during mitotic arrest and/or an enhanced cell capability to escape from mitotic arrest and to give rise to polyploid cells, contribute to resistance. In this review, we specifically focus on recent findings which indicate that BCL2 family members govern the survival of cancer cells during mitotic arrest, as well as that of polyploid cells that have escaped mitosis, thereby providing a rationale for the use of their inhibitors in combination with anti-mitotic agents.

BCL2 Family Members as Actors and Targets of Life/Death Decisions During Prolonged Mitotic Arrest

Mitotic arrest corresponds to a very specific state which is understood to impact on the BCL2 network. In most experiments, cell death induced by anti-mitotic drugs is indeed not only caspase-dependent but also BAX- and/or BAK-dependent and results in MOMP (5, 6). Accordingly, BCL2 overexpression enhances anti-mitotic resistance, at least partially and transiently (5). Increased expression of anti-apoptotic BCL2 proteins or decreased expression of proapoptotic BH3-only proteins has been reported in cancer cells selected for taxane-acquired resistance. In the latter case, treatment with the BH3 mimetic ABT-737 could restore sensitivity to taxanes (7). Thus, modifications in the BCL2 network induced by anti-mitotic drugs are likely to play a critical role in determining whether prolonged mitotic arrest (whether it is transient or not) will kill or spare the treated cells. Which cellular events trigger these modifications? Cells treated with anti-mitotic drugs exhibit a pro-metaphase delay due to activation of the spindle assembly checkpoint (SAC), which monitors defects in microtubule-kinetochore attachment, and the tension of mitotic spindles. This checkpoint is usually weakened in tumor cells and they are often less able to maintain it so that even though tumor cells usually arrest in response to anti-mitotic drugs, this arrest is transient and cells slip out of mitosis without division (a process called mitotic slippage). Long-term treatment with spindle inhibitors can thus have several outcomes: (i) chronic arrest in mitosis until the drug is removed; (ii) cell death during mitotic arrest; (iii) mitotic slippage corresponding to an adaptation from mitosis into G_1 without cytokinesis, leading to tetraploidy, apoptotis, senescence or continued cycling (8). A failure to destroy cells that have slipped out of mitosis from the cycling population may allow for cells to continue proliferating with an abnormal number of chromosomes. Cell death during mitotic arrest and apoptosis or senescence following mitotic slippage are, thus, crucial for eliminating such cells and preventing chromosome instability.

One hallmark of mitotic arrest is prolonged activity of cyclin-dependent kinase 1 (CDK1). During normal mitosis, CDK1 activity is tightly regulated through various mechanisms but the level of its activator, cyclin B1 (which rises then declines during mitosis onset), governs CDK1 kinase activity. The mitotic checkpoint, which can be satisfied only when chromosomes are correctly attached to microtubules, mainly acts through the regulation of cyclin B1 degradation, by triggering its ubiquitylation by the E3 ubiquitin ligase anaphase-promoting complex/cyclosome (APC/C) and targets it to the proteasome. This latter event reduces CDK1 activity and drives cells out of mitosis (8). The cyclin B1-CDK1 complex coordinates the phosphorylation cascade of various substrates. Among them are some BCL2 homologs, such as BCL2, BCLXL and MCL1.

The consequences of MCL1 phosphorylation by CDK1, during prolonged mitosis, have been extensively studied. It determines MCL1 ubiquitylation and its further proteosomal degradation through modifications by various E3 ubiquitin ligases (Figure 1). CDK1 phosphorylates MCL1 on Thr92, this makes it sensitive to polyubiquitylation by the APC/C (9). It also prevents interaction with the protein phosphatase 2A (PP2A), and favors phosphorylation at Ser121 by c-jun N-terminal kinase (JNK) or p38. The latter phosphorylation targets MCL1 to polyubiquination by a process that relies on the E3 ubiquitin ligase F-box and WD repeat domain containing 7 (FBW7), a haploinsufficient tumour suppressor that also targets proto-oncoproteins such as c-MYC, c-JUN, Notch and cyclin E for degradation (10). Another E3 ubiquitin ligase, MULE/HUWE1 may contribute to MCL1 degradation during mitotic arrest but the mechanisms involved have not been defined (11).

CDK1 was also shown to phosphorylate BCL2 and BCLXL. During normal mitosis CDK1 transiently and incompletely phosphorylates these substrates but when mitosis is prolonged, *e.g.* in the presence of microtubule inhibitors, BCL2 and BCLXL become highly phosphorylated on multiple serine/threonine sites, without being degraded as in the case of MCL1 (12). CDK1 is responsible for BCL2 phosphorylation at the Thr56 residue, which can be detected on mitotic chromosomes or in the mitochondrial fraction (13,

14), and for BCLXL phosphorylation on Ser62 (12, 15). Phosphorylation sites stand within the nonstructural loop that links the BH3 and BH4 domains of these proteins (*i.e.* Thr56, Thr69, Ser70, Ser87 for BCL2), and they are not directly part of the BH3 binding. Their effect on the antiapoptotic activity of BCL2 and BCLXL are thus not completely understood. It was nevertheless established that Ser62 phospho-defective BCLXL mutants are unable to release BAX in response to anti-mitotic drugs and that they efficiently protect cells from mitotic arrest-induced apoptosis (12, 15), suggesting that mitotic phosphorylation of BCLXL erodes its antiapoptotic activity. As in the case of MCL1, JNK, p38 but also kinases of the ERK pathway and polokinases (PLK), were shown to contribute to hyperphosphorylation of BCL2 and BCLXL during mitotic arrest (16-20).

The view that emerges from the above data is that phosphorylation of antiapoptotic members of the BCL2 family by CDK1 or other kinases during prolonged mitosis, tips the balance towards cell death (Figure 2). These findings suggest a model whereby a switch in the duration of CDK1 activation dramatically increases the extent of BCL2, BCLXL and MCL1 phosphorylation, which acts as a sensor for CDK1 signal duration, resulting in the loss of their anti-apoptotic function, thus coupling mitotic arrest to apoptosis. However active CDK1 has an ambiguous effect on apoptosis since it may simultaneously promote cell survival by inhibiting the pro-apoptotic activity of caspase-9 (21) or by stabilizing survivin, which indirectly affects the caspase cascade (22). Thus, CDK1 may promote a type of cell death that relies on MOMP but in which caspase activation is somehow uncoupled from this permeabilization process. This may permit cell recovery after mitotic slippage by limiting cell damage during prolonged mitosis. Such recovery may also be allowed by the reversion of BCL2 homologs phosphorylation by phosphatase activity. In addition to PP2A de-phosphorylation of MCL1, the protein phosphatase 1 (PP1) was shown to de-phosphorylate BCL2 during mitotic slippage (13, 23), possibly reactivating its anti-apoptotic function. Likewise, ubiquitinylation of MCL1 is a reversible process: the de-ubiquitinase USP9X (24) promotes cell survival by removing Lys48-linked polyubiquitin chains that otherwise target MCL1 for proteasomal degradation. Mitotic arrest is also characterized by high energy consumption and by nuclear envelope breakdown (that occurs at mitosis onset). Various cellular processes such as gene transcription and canonical cap-dependent protein translation are thus shut down or altered. The rapid decline of MCL1 during mitosis is not only due to CDK1 phosphorylation-dependent induction of its polyubiquitylation but also to the short half-lives of the corresponding mRNAs. In contrast, both long half-lives and the presence of a putative internal ribosomal entry site element (IRES) in *BCL2* and *BCLXL* mRNAs may contribute to preserving stable protein expression levels during prolonged mitosis by allowing cap-independent translation (25). The IRES-transactivating factor DAP5 was shown to protect cancer cells from undergoing apoptosis during mitosis by maintaining BCL2 synthesis, together with that of CDK1 (26). The endogenous caspase inhibitor X-linked inhibitor of apoptosis protein (XIAP) probably also contributes to mitotic survival through its IRES-mediated translation (27). Notably, a screen for regulators of alternative splicing of BCLX and MCL1 (which favor the expression of shorter, proapoptotic forms) identified many cell cycle factors involved in mitotic progression (28). As the splicing regulation unraveled by these studies necessarily precedes mitotic entry, this puts forth the notion that cells may anticipate abnormal mitosis by mounting pro-death events before mitotic onset. This notion is strengthened by the observation that p73, the functional homolog of p53, induces BH3-only *BIM* gene transcription before entry in mitosis (29). Thus, *via* an intricate link between cell cycle control and apoptosis, cells may be naturally primed to mitotic death by predetermined signals that spare cells if the duration of mitosis is appropriate, and kill them when mitosis lasts longer than a critical period of time. As seen above, BCL2 family members are crucial regulators of cell death upon mitotic arrest since BCL2 overexpression, phospho-defective BCLXL mutants and MCL1 stabilization strongly delay this process. This tends to suggest that mitotic cell death occurs in great part through the mitochondrial apoptoptic pathway. However, caspase activation downstream of MOMP may be inhibited by CDK1 phosphorylation of caspase-9, stabilization of survivin and/or maintenance of XIAP expression. This may explain why death during prolonged mitosis has sometimes been shown to occur by non-canonical pathways, and to be caspase independent (30). Our knowledge in this field needs to be deepened to unravel how cell death pathways interconnect during mitotic arrest, and whether and how BCL2 family members control one or more of these pathways.

BCL2 Family as Regulators of Mitotic Slippage, and of Survival Afterwards

Gascoigne and Taylor proposed a new model to account for mitotic slippage, in which two alternative pathways compete with each other. One, the mitotic death pathways promotes cell death during prolonged mitosis, and the other, the exit pathway promotes cyclin B1 degradation and mitotic exit. Cyclin B1 levels mainly govern CDK1 activity during mitosis and during a normal mitosis, the active E3-ligase complex APC/C drives cyclin B1 to proteasome degradation, leading to decreased CDK1 activity and to mitotic exit. During prolonged mitotic arrest, cyclin B1 levels are more stable but, by slow degradation, they may nevertheless fall under the threshold necessary to maintain sufficient CDK1 activity, allowing for cells to escape from mitotic arrest without completing

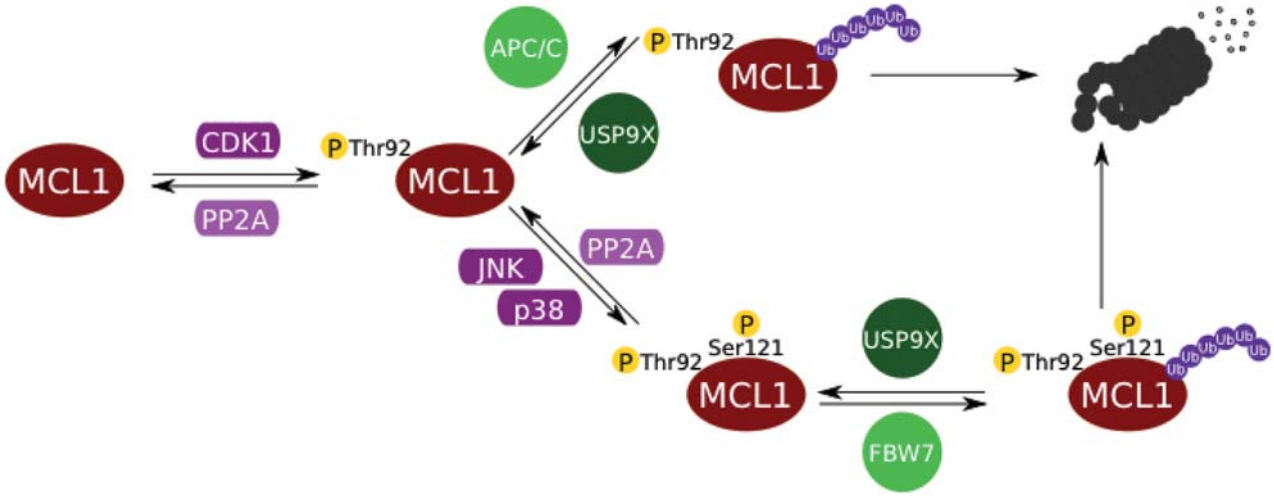

Figure 1. *Post-translational modifications that regulate MCL1 level during mitotic arrest. Upon mitotic arrest, cyclin-dependent kinase 1 (CDK1) phosphorylates the induced myeloid cell leukemia differentiation protein (MCL1) on Thr92, this makes it sensitive to polyubiquitylation by the E3 ligase anaphase-promoting complex/cyclosome (APC/C). It also prevents its interaction with the protein phosphatase 2A (PP2A), and favors its phosphorylation at Ser121 by JNK or p38, that targets MCL1 to polyubiquination by the E3 ubiquitin ligase F-box and WD repeat domain containing 7 (FBW7). Polyubiquitylation by APC/C or FBW7 drives MCL1 to proteosomal degradation. In the opposite reaction, the de-ubiquitinase USP9X protects MCL1 from degradation.*

cytokinesis. Kinetics and the thresholds of both pathways govern cell survival and probably determine cell fate (5). To model mitotic death and slippage, there is, *a priori*, no need to believe that the above pathways are connected: blocking mitotic slippage is expected to promote mitotic cell death by allowing the accumulation of mitotic death signals, whereas conversely, preventing cell death during mitosis may favor exit by giving cells time to degrade cyclin B1 (31). According to this scheme, BCL2 homologs may favor mitotic slippage solely by acting as survival proteins during prolonged mitosis. Conversely, slow cyclin B1 degradation may only influence exit from mitosis without impacting on mitotic death signals. However, two lines of evidence indicate that this model needs to be refined. Firstly, as shown above, prolonged CDK1 activity modifies key regulators of cell death. We, thus, propose a novel model to account for mitotic death and slippage, in this model, mitotic death signals, CDK1 activity and time spent in mitosis are linked, and not independent, variables (Figure 2). Whereas CDK1 activity would linearly decrease with respect to time spent during mitosis (as a direct result of cyclin B1 degradation), the intensity of death signals during mitosis would result from the integration of CDK1 activities over time. Death signals would, thus, be linked to time spent in mitosis not linearly but by a quadratic function. As a result, they may first increase quickly before declining, at a time point that depends on the intensity of death signals and of CDK1 activity at the onset of mitosis, and on the rapidity with which CDK1 activity declines over time. This model predicts that a cell that is prone to escape from mitosis (*i.e.* that rapidly degrades its cyclin B1), will accumulate fewer death signals for the same time spent in mitosis. Secondly, survival molecules can, conversely, regulate mitotic exit. Indeed, some reports indicate that the mitotic death and the escape pathways have some molecules in common. For instance, even though mitotic slippage and caspase activity seem not to be linked (32), the mitotic checkpoint regulator BUBR1 was identified as a caspase substrate (33). Moreover, some BCL2 homologs themselves may be involved in regulating mitotic exit directly. Ser62-phosphorylated BCLXL may interact with the APC/C inhibitory complex during mitotic checkpoint and the phopshorylation of its Ser49 residue by polokinase (PLK) 3 modulates mitosis progression to cytokinesis (20). In addition, since CDK1 detectably interacts with its substrates BCL2 and BCLXL in mitotic cells, the latter proteins may reciprocally impact on CDK1 activity (13, 34). The consequence of this interaction on mitosis duration remains to be defined, but it underscores that BCL2 homologs may promote mitotic slippage not only as survival proteins, but also as regulators of mitotic progression. Mitotic slippage corresponds to an abnormal exit from mitosis without cell division that generates tetraploid or polyploid cells in a pseudo-G_1 phase. An elegant analysis performed by *in vivo* microscopy on xenografts showed that in response to anti-mitotic drugs, few tumor cells died directly from mitotic arrest but most of them survived longer after mitotic arrest and became multinucleated (35). By favoring mitotic slippage upon treatment with anti-mitotic drugs, BCL2 homologs may thus favor the emergence of polyploid cells. Consistent with this, decreased MCL1

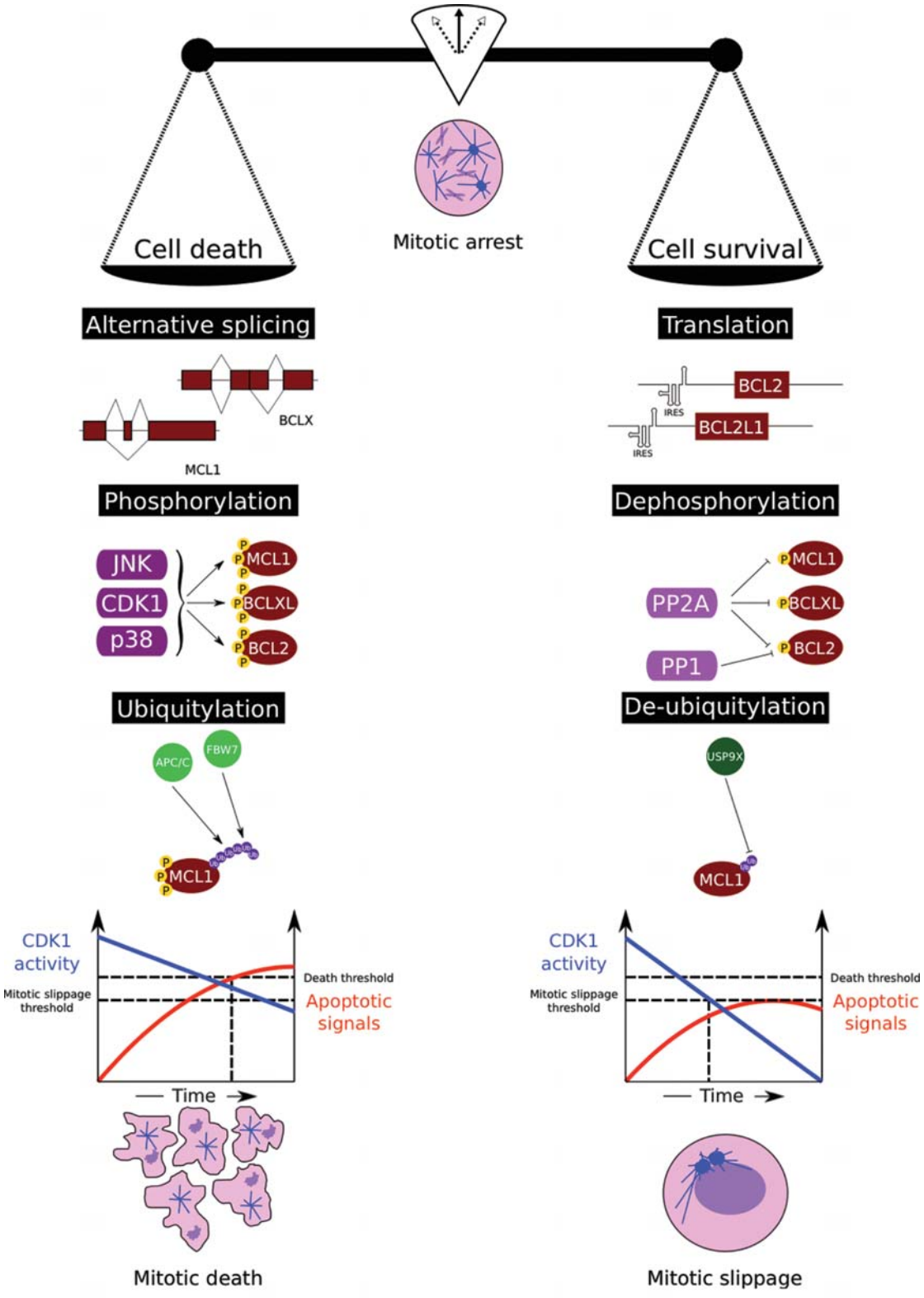

Figure 2. *Regulation of the BCL2 family antiapoptotic members on the survival/cell death balance during mitotic arrest. Proapoptotic alternative splicing and antiapoptotic internal ribosomal entry site element (IRES)-dependent translation combined with phosphorylation/dephosphorylation and ubiquitylation/de-ubiquitylation regulate the levels of BCL2 homologs during mitotic arrest. In modulating both mitotic exit and apoptotic threshold, levels of active cyclin-dependent kinase 1 (CDK1) determine mitotic cell fates.*

degradation through decreased FBW7 activity increases anti-mitotic drug-induced polyploidy (10). Are BCL2 homologs required to maintain the survival of polyploid cells? The destruction of such cells is of key importance because the aberrant capacity of polyploid cells to survive in overloaded stress conditions is a prominent source of aneuploidy in cancer: even though polyploid cells spawn viable progeny far less frequently than diploid cells, polyploidy increases chromosome instability and may even lead to a de-polyploidization process (36). Mitotic slippage is essentially a survival process but it is accompanied by the appearance of a cell response that can lead to senescence or apoptosis during the subsequent interphase due to the activation of an ill-characterized checkpoint. It is envisioned that this cell response is initiated during mitotic arrest, due to damage accumulation, and that its execution is delayed because it can be effective only after mitotic slippage, when, for instance, caspase-9 is de-phosphorylated and canonical cap-dependent protein translation is restored (35). Importantly, triggering of the mitochondrial apoptotic pathway by the p53 transcription factor may contribute to post-mitotic cell death, since *p53* and *BAX* knock-out cells allow accumulation of tetraploid cells after treatment with anti-mitotic drugs (37). Regardless of the exact nature of the post-mitotic death signals, a key aspect of the polyploidization process is that it renders cellular adaptations necessary for the survival of cells with an abnormal number of chromosomes (38). BCL2 family members may play a pivotal role in this process. Indeed, a specific addiction to BCLXL has been observed in polyploid cancer cells obtained by cytokinesis inhibitors such as aurora-B inhibitors, and its neutralization but not that of BCL2 or MCL1, elicits cell death of these cells. In this case, the burden of supporting viability has shifted from MCL1 to BCLXL as polyploid cells accumulate NOXA by uncharacterized mechanisms (39). Thus, anti-mitotic treatments may produce tetraploid/polyploid tumor cells that have escaped from mitotic death and have adapted to survival by raising their apoptotic threshold despite sustained checkpoint activation. The frequency of this process and how it occurs remains to be characterized, but BCL2 family members may intervene at multiple levels: by allowing survival during mitotic arrest, by favoring mitotic escape, and by allowing polyploid cells to survive.

Therapy: New Avenues and Key Questions

The anti-mitotic agents currently used interfere with microtubule dynamics and have shown great success in anticancer therapy. But two factors have limited their effectiveness, namely resistance and toxicity. Thus, enhancing their efficacy is critical. Cell line-based experiments predict that sustaining mitotic arrest triggers apoptosis more efficiently. Since cancer cells often have a leaky mitotic checkpoint and could be thus prone to escape mitotic arrest before apoptotic signaling reaches an effective threshold, inhibiting mitotic slippage could therefore enhance the efficacy of anti-mitotic drugs. New mitotic targets besides tubulin have been, thus, characterized to be exploited in anticancer treatment. Knock-down of APC/C co-activator cell division cycle (*CDC*) 20 potently reduced mitotic slippage and significantly promoted cancer cell death (40). This strategy has led to tumor regression in a mouse model (41). A pharmacological inhibitor of APC/C also promoted strong mitotic arrest without SAC leakage and induced a potent apoptotic effect (42). The mitotic kinesin spindle protein (KSP), which is critical for proper spindle assembly, is also a relevant mitotic target whose inhibition leads to mitotic arrest (43). Moreover, mitotic kinases such as aurora or PLK have also emerged as mitotic targets for cancer therapeutics. A wide array of innovative mitotic targets has emerged from pre-clinical experiments in the past decade. Unfortunately, few of these compounds have shown as much antitumor activity as paclitaxel in clinical trials (44) and their use in cancer therapy has to be reconsidered. From the discussion above, another rational approach is to combine currently used anti-mitotic drugs with pro-apoptotic compounds. Upon anti-mitotic treatment, a fine balance between pathways that control cell death and mitotic slippage will finally govern cell fates. As BCL2 homologs critically regulate cell death upon mitotic arrest, inhibitors of the anti-apoptotic activity of these proteins could be particularly effective in enhancing anti-mitotic pro-death activity. Since the crystal structure of BCLXL was determined, various approaches have focused on inhibiting BCL2/BCLXL function with small molecules. Using structure-activity relationships, Abbott Laboratories ultimately produced the potent BH3-mimetic molecule ABT-737 and its orally active form ABT-263 (or navitoclax) which is currently being tested in clinical trials against cancer. These molecules have close structural or functional similarity to BH3-only proteins and bind BCL2, BCLXL and BCLw BH3 pocket with high affinity, but exhibit low affinity for MCL1 and A1/BFL1. These molecules have demonstrated killing potency in combination settings with diverse chemotherapeutic agents in cell line-based models and in primary xenografts or in primary cancer cells. They trigger BAX/BAK-dependent cell death in cancer cells (45), but one limitation of these compounds is that they do not inhibit MCL1, whose overexpression in cancer cells can lead to ABT-737/263 inefficacy (46, 47).

Importantly, these drugs have recently been shown to potentiate the cytotoxic effect of paclitaxel in cell cultures and in animal models. Indeed, on analysis by videomicroscopy using *in vitro* cancer cell lines (11), and a mouse xenograft model (48), addition of ABT-263 to paclitaxel was shown to change cell fate from mitotic slippage to death during mitotic arrest. This suggests that the ability of BCL2 and BCLXL to regulate cell survival during

mitotic arrest, and possibly their ability to regulate mitotic escape, can be harnessed pharmaceutically. In line with this, tumor response and overall survival were significantly improved in therapy combining ABT-737 and docetaxel for human breast basal-like tumor xenografts (49). This was particularly meaningful for tumors that expressed elevated levels of BCL2 where ABT-737 probably sensitized tumor cells to doxetaxel in dissociating BIM from BCL2 and in activating downstream apoptotic signaling through BAX- and BAK-dependent pathways. While the above strategies are based on the notion that BH3-mimetics might enhance the probability of cells undergoing cell death during mitosis, it is nevertheless unclear, from the *in vivo* assays reported above whether BH3-mimetics enhance mitotic cell death or another form of cell death. Because of their neurotoxicity, anti-mitotic drugs are administer in bolus and even though some breast tumors showed a modest increase in mitotic index and little cell death in the first 48 h after paclitaxel treatment, these tumors later converted to a sustained apoptotic response by unknown mechanisms (50). For practical reasons, it is thus this type of response that needs to be amplified by sensitizing compounds, and BH3-mimetics in particular. It is also important to mention here that BH3-mimetics would be especially useful in eradicating polyploid cells that have escaped from taxane-induced mitotic arrest (39). Abnormal mitosis induced by anti-mitotic drugs may lead cancer cells to an apoptosis-prone polyploid state that could be beneficially targeted by a BH3-mimetic. Because cell fates upon treatment with anti-mitotic drugs result from a complex network of tightly interconnected signaling pathways, it is particularly important to define approaches that can predict the efficacy of anti-mitotic drugs on cancer cells. Numerous gene expression signatures have been derived with the aim of identifiying pharmacogenomic predictive markers (51, 52). These signatures are nevertheless mostly derived from studies performed on cell lines, where all cells are cycling and where the influence of the tumor microenvironment on cancer cell response is not taken into account. We have developed a 3D short-term *ex vivo* model in which the architectural integrity of the tumor, including its microenvironment, is preserved. This model provides a powerful, rapid and reproducible tool for studying the differential responses of individual breast tumors (and their various components) to specific treatments (53), for classifying tumors based on their response to one given treatment and for identifying genes or proteins whose expression discriminates sensitive from resistant tumors. Interestingly, preliminary experiments using this model point to a significant synergy between paclitaxel and ABT-737 in terms of apoptosis induction (personal data). An alternative functional cell assay has been developed by Chonghaile and colleagues (54) that can predict the usefulness of BH3-mimetics. This assay is based on the postulate that sensitivity to induction of apoptosis relies in great part on the existence, at the mitochondria, of protein complexes between pro- and anti-apoptotic members of the BCL2 family. Thus, a BH3 profiling assay which measures the mitochondrial response of tumor cells to a panel of peptides derived from pro-apoptotic BH3 domains might help to quickly identify tumors that would benefit from pro-apoptotic activity. Since the death signaling pathways of anti-mitotic agents converge towards the intrinsic cell death pathway, BH3 profiling is expected to indicate which tumors would respond to anti-mitotic agents used alone or together with BH3-mimetics. Notably, this assay was shown to be indicative of a clinical response to cytotoxic chemotherapy (54).

Conclusion

Intensive apoptosis research has demonstrated that shifting the balance of BCL2 family rheostat towards pro-apoptotic members in neutralizing anti-apoptotic members provides a powerful means of promoting cell death and improving chemotherapy efficiency. In addition, comprehensive research on mitosis has considerably increased our understanding over why cell responses to anti-mitotic drugs are variable. These data, plus pre-clinical data which revealed that combining anti-mitotic drugs with BH3-mimetics strongly accelerates apoptosis during mitotic arrest, point to exciting potential for the treatment of cancer.

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