Review

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Mitosis and mitochondrial priming for apoptosis

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Abstract: Cell division is a period of danger for cells, as inaccurate segregation of chromosomes can lead to loss of cell viability or aneuploidy. In order to protect against these dangers, cells ultimately initiate mitochondrial apoptosis if they are unable to correctly exit mitosis. A number of important chemotherapeutics exploit this response to delayed mitotic exit, but despite this, the molecular mechanism of the apoptotic timer in mitosis has proved elusive. Some recent studies have now shed light on this, showing how passage through the cell cycle fine-tunes a cell's apoptotic sensitivity such that it can respond appropriately when errors arise.

Keywords: apoptosis; Bcl-2 proteins; mitochondrial priming; mitosis.

Introduction

Proliferation exposes the dividing cell to significant dangers. Errors in DNA replication or during the segregation of chromosomes in mitosis can result in non-viable daughter cells, or allow the acquisition of oncogenic mutations (Hartwell, 1992). In multicellular organisms, increased proliferation is a key molecular event in the transformation into cancer (Hanahan and Weinberg, 2011). To avoid deleterious errors, cell cycle progression is regulated by checkpoints that allow the cell to advance only if it has successfully completed each stage. Failure to satisfy the checkpoint requirements causes the cell to arrest, allowing damage to be repaired. If the damage cannot be repaired, the cell will activate apoptosis (Topham and Taylor, 2013). These connections between cell cycle progression and the apoptotic regulators represent one of the cornerstones for chemotherapeutic intervention in cancer. Many cytotoxic treatments are aimed at exploiting the inbuilt sensitivity of cells that is established by cell cycle checkpoints (Dumontet and Jordan, 2010; Roos et al., 2016). As many of the key regulators of these checkpoints function as oncogenes or tumour suppressors, it is clear that close coordination of proliferation and apoptosis is a fundamental aspect of cell cycle.

In mitosis, the cell ensures that each daughter receives the correct allocation of genomic material, delaying the transition to anaphase until replicated chromosomes are correctly aligned and attached to the spindle (Lara-Gonzalez et al., 2012). If a cell remains in mitosis for longer, whether it undergoes death in mitosis (DiM) is determined by members of the Bcl-2 family of apoptosis regulators (Kutuk and Letai, 2008). Bcl-2 proteins act at the surface of mitochondria to control mitochondrial outer membrane permeabilisation (MOMP), the defining point of the intrinsic apoptotic pathway (Tait and Green, 2010).

Mitochondrial priming describes how close a cell is to undergoing MOMP (Certo et al., 2006). Priming is a critical determinant for whether or not a cell will respond to an apoptotic signal at a given point in time. How 'primed' a cell is will be determined by the spectrum of expression, localisation and activation of Bcl-2 proteins, all of which can alter in response to internal and external signals. This allows cells to constantly adapt their apoptotic landscape as they progress through each checkpoint, adjusting their apoptotic sensitivity appropriately. This review will discuss mitochondrial priming and examine recent evidence showing how this is coordinated by changes in Bcl-2 family proteins during mitosis.

Bcl-2 proteins – controlling mitochondrial permeabilisation

Apoptosis can be initiated by either the extrinsic or intrinsic pathways. The extrinsic pathway involves ligand-binding to transmembrane receptors of the tumour necrosis factor receptor (TNF-R) superfamily, resulting in the direct

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activation of initiator caspases-8 and -10 (Green and Llambi, 2015). The intrinsic pathway is focused on the Bcl-2 family, which functions largely on the outer mitochondrial membrane to initiate MOMP (Youle and Strasser, 2008). MOMP results in the passive release of cytochrome *c* and second mitochondria-derived activator of caspases (SMAC)/Diablo from the mitochondrial intermembrane space, which then coordinate the activation of initiator caspase-9. Following MOMP, the kinetics of cell death are rapid.

Bcl-2 proteins regulate mitochondrial permeability through interactions between members of three distinct sub-groups within the family (Leber et al., 2007; Youle and Strasser, 2008). These groups of Bcl-2 proteins are defined by both their role within the apoptotic pathway and the number of Bcl-2 sequence homology (BH) domains they possess. Bax and Bak comprise one class of Bcl-2 proteins that act as pro-apoptotic effector proteins. When activated, these BH1–3-containing proteins oligomerize and form pores in the mitochondrial outer membrane (Antonsson et al., 2001; Wei et al., 2001; Annis et al., 2005). The anti-apoptotic BH1–4 containing Bcl-2 proteins function to repress Bax and Bak activation, inhibiting MOMP. This class includes Bcl-2, Mcl-1, Bcl-XL and Bcl-W. The third class are the BH3-only proteins, containing only the one region of homology (Happo et al., 2012). BH3-only proteins are pro-apoptotic, but unlike Bax and Bak, are unable to bring about MOMP by themselves. Bid, Bim, Bad, Puma and Noxa belong to this BH3-only class, and are regulated in response to a variety of cellular stresses such as DNA damage (Villunger et al., 2003; Michalak et al., 2008) or cytokine deprivation (Bouillet et al., 1999; Gilmore et al., 2002; Herold et al., 2014).

The BH3 domain of the BH3-only proteins is the key functional motif that mediates their interaction with both the pro- and anti-apoptotic multi-BH domain Bcl-2 proteins (Letai et al., 2002). The specificity of BH3 domain-binding varies between BH3-only proteins. Some are promiscuous, such as Bid and Bim. Others, such as Bad, are more restricted, and cannot interact with pro-apoptotic Bax and Bak. NOXA appears only to bind to Mcl-1 and Bcl-A1. Those BH3-only proteins that interact directly with Bax and Bak can induce MOMP, and are termed direct activators. Those that only bind the anti-apoptotic BH1–4 proteins, such as Bad and NOXA, are termed sensitisers (Letai et al., 2002; Kim et al., 2006). Cells can express a bewilderingly complex spectrum of Bcl-2 proteins. The sum of the interactions between these proteins ultimately determines whether Bax and Bak activate, resulting in MOMP (Lovell et al., 2008). How these interactions induce MOMP in cells has been a controversial point over the years, becoming polarised between the 'direct activation' and 'derepression' models (Kim et al., 2006; Willis et al., 2007) (summarised in Figure 1). However, recent studies have suggested that these represent two extremes of a spectrum of how a cell can be poised to respond to an apoptotic signal (Llambi et al., 2011). How a cell is poised with regard to apoptosis is termed 'priming'.

Apoptotic priming – dynamically setting the threshold for MOMP

A conceptual advance to understanding the response of cells to apoptotic stimuli is how the Bcl-2 profile of a cell determines how close to MOMP mitochondria is, referred to as mitochondrial priming (Certo et al., 2006). Mitochondrial priming can be measured by BH3-profiling, whereby cells are exposed to synthetic peptides mimicking the BH3-domains of different BH3-only proteins (Certo et al., 2006; Deng et al., 2007; Chonghaile et al., 2011). If a cell has an abundance of pro-apoptotic Bcl-2 family proteins residing on its mitochondria, then exposure to BH3 peptides will result in MOMP and loss of mitochondrial membrane potential, which can be easily measured. Such cells are considered to be 'primed', and are exquisitely sensitive to an apoptotic stimulus. Conversely, cells can be 'unprimed', either through overexpression of anti-apoptotic Bcl-2 proteins or reduced expression of the pro-apoptotic ones (Figure 2). Exposure of unprimed cells to BH3 peptides or an apoptotic stimulus may not result in MOMP. The degree of mitochondrial priming varies and is influenced by cell context, such as tissue type or differentiation status. For example, differentiated brain and kidney cells are unlikely to die when exposed to an apoptotic stimulus, while undifferentiated embryonic stem cells readily undergo apoptosis (Chonghaile et al., 2011; Vo et al., 2012; Liu et al., 2013). BH3 profiling applied to primary tumour cells indicates that in many cases these are already primed. Such analysis of tumour cells freshly isolated from patients can predict the clinical response of that patient to chemotherapy, as primed tumour cells are more sensitive to apoptosis when exposed to chemotherapeutic drugs (Chonghaile et al., 2011; Montero et al., 2015).

The binding specificity of the different BH3 peptides allows the functional profile of anti-apoptotic Bcl-2 proteins within a cell type to be determined. The relevance of this is that BH3 mimetics, such as navitoclax, can be used to increase mitochondrial priming in cancer cells (Colak et al., 2014). However, the specificities of these mimetics mean that only cells dependent upon particular anti-apoptotic Bcl-2 proteins are sensitive. Thus, a cell dependent on

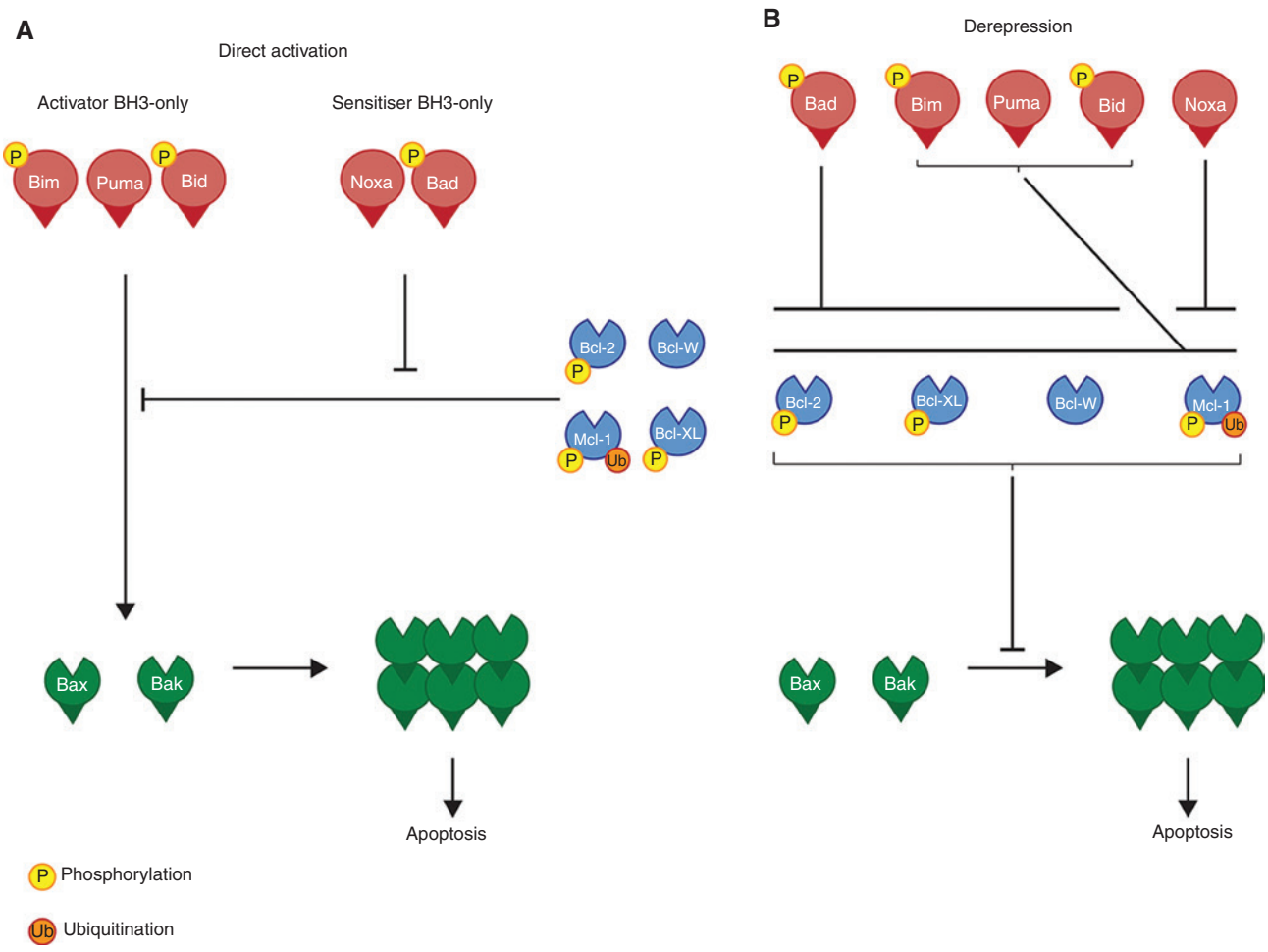

Figure 1: Models for regulation of mitochondrial apoptosis.
Current consensus is that aspects from both direct activation and derepression act in unison to regulate the induction of apoptosis. Key proteins known to undergo mitosis-specific post-translational modifications are highlighted. (A) Direct activation: BH3-only proteins exist in two distinct sub groups know as 'activators' and 'sensitisers'. Activator BH3-only proteins are able to directly bind and activate Bax and Bak, inducing their oligomerisation and the permeabilisation of the mitochondrial outer membrane. Anti-apoptotic BH1–4 proteins function by binding and sequestering the direct activator BH3-only proteins, thus preventing them from activating Bax and Bak. BH3-only proteins that do not directly interact with Bax and Bak act as 'sensitisers', able to displace the activators that have been sequestered by anti-apoptotic proteins. (B) Derepression: Bax and Bak are constitutively active on the mitochondrial outer membrane; however, anti-apoptotic Bcl-2 proteins bind to Bax and Bak preventing them from oligomerising, thus preventing MOMP and apoptosis. BH3-only proteins are able to bind to anti-apoptotic proteins and displace Bax or Bak, allowing them to permeablise the mitochondrial outer membrane.

Bcl-XL or Bcl-2 will respond to navitoclax, a Bad mimetic, but a cell dependent on Mcl-1 will not (Oltersdorf et al., 2005; Konopleva et al., 2006; Tahir et al., 2007; Shi et al., 2011). Colon cancer stem cells, for example, are selectively resistant to chemotherapeutic agents such as oxaliplatin because of a reduction in priming due to Bcl-XL, relative to differentiated cells (Colak et al., 2014). However, these cancer stem cells could be re-sensitised with Bcl-XL-specific BH3 mimetics.

Mitochondrial priming is a dynamic property, altering with changes in intracellular signalling. Oncogenic signalling often results in a reduction in priming due to these pathways impacting on the activity or expression of Bcl-2 family proteins. Inhibiting these oncogenic pathways with targeted drugs may not directly induce apoptosis, but may increase mitochondrial priming and, thus, sensitivity to other more conventional chemotherapies. In a recent study, treating primary tumour cells with a range of targeted drugs, followed by BH3 profiling, was able to identify those drugs that increased mitochondrial priming (Montero et al., 2015). This increase in priming *in vitro* might be used to predict clinical response.

As apoptosis occurs at a single-cell level, variations in priming occur at the single-cell level as well. Single-cell

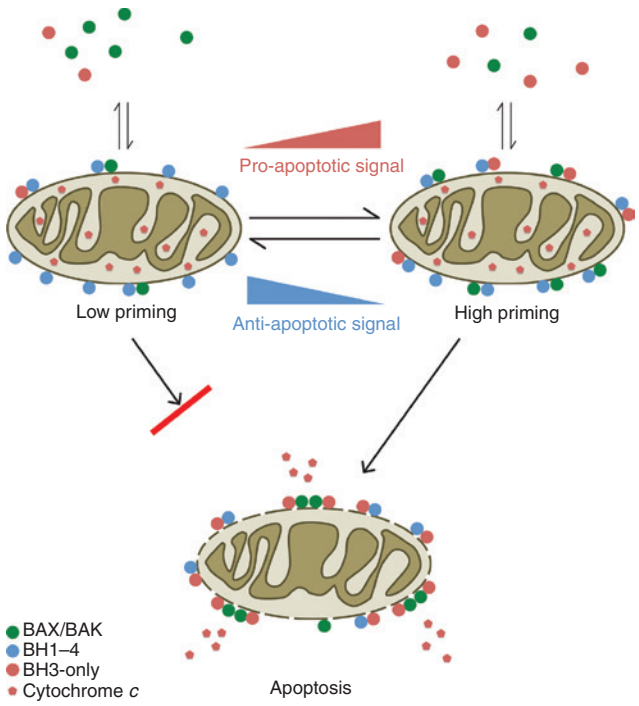

Figure 2: Fluctuations in Bcl-2 populations on the mitochondrial outer membrane determine apoptotic priming.
Cells are able to fluctuate between states of high and low priming by changes in expression and localisation of pro- and anti-apoptotic Bcl-2 family. An unprimed cell may have an excess of anti-apoptotic BH1–4 proteins on mitochondria, while the majority of pro-apoptotic proteins may reside in the cytosol or be expressed at low levels. By increasing the expression of BH3-only proteins, or by shifting the population of pro-apoptotic proteins to the mitochondria, the cell can increase apoptotic priming, bringing itself closer to mitochondrial permeabilisation and making itself more sensitive to further apoptotic signals. Conversely, priming can be reduced by destabilising pro-apoptotic proteins from the mitochondrial membrane, reducing the expression of pro-apoptotic BH3-only proteins, and returning to an excess of an anti-apoptotic Bcl-2 family at the mitochondria.

heterogeneity in apoptosis is a long-standing observation. Live-cell imaging of cell populations treated with an apoptotic stimulus show variations in both the proportion of cells that respond and the time required before caspase activation is observed in the responders (Rehm et al., 2002; Niepel et al., 2009). The events following caspase activation, however, occur with consistent kinetics. For example, cells exposed to TNF or TNF-related apoptosis-inducing ligand (TRAIL) initiate signalling immediately downstream, in this case, caspase-8 (an initiator caspase) activation, but the onset of caspase-3 activation is heterogeneous. This variability is dependent upon a range of factors, including expression of an X-linked inhibitor of apoptosis protein (XIAP, a caspase inhibitor), the BH3-only protein Bid, and time to onset of MOMP (Albeck et al., 2008; Roux et al., 2015). Daughter cells were seen to die simultaneously if apoptosis occurred soon after cell division, but this effect was rapidly lost with increasing time following division (Spencer et al., 2009). Furthermore, cell populations treated with staurosporine or TNF/cylcohexemide tend to have a proportion of sensitive and resistant cells. If the resistant cells are propagated and then challenged with the same stimulus, then the proportion of sensitive to resistant cells is often similar to when they were originally exposed (Flusberg et al., 2013). Together, this indicates that non-genetic variations in priming contribute to the heterogeneity of apoptosis seen within a cell population.

A predominant view of pro-apoptotic Bcl-2 proteins is that they reside in a silent, inactive state prior to being switched on to form oligomeric pores (Gavathiotis et al., 2010). In particular, Bax is thought to reside in the cytosol of a healthy cell and to translocate to mitochondria following its activation by BH3-only proteins like Bid and Bim. A number of recent studies have indicated that this is not the case. Using live-cell imaging approaches, GFP-Bax was found to constitutively target mitochondria, but in healthy cells was rapidly retrotranslocated back to the cytosol (Edlich et al., 2011; Schellenberg et al., 2013). Epithelial cells are exquisitely sensitive to anoikis: apoptosis initiated by loss of adhesion to the extracellular matrix (ECM) (Gilmore et al., 2000). Loss of ECM contact in epithelial cells results in the rapid accumulation of Bax on mitochondria, prior to any detectable MOMP (Valentijn et al., 2003). If these cells are then allowed to reattach to ECM, Bax redistributes back to the cytosol. This rapid alteration in Bax distribution, over a few minutes, is controlled by changes in kinase signalling downstream of integrins. As survival signalling was inhibited in these anoikis-sensitive epithelial cells, Bax retrotranslocation slowed and it accumulated on mitochondria (Schellenberg et al., 2013). Reducing retrotranslocation allowed Bax accumulation on mitochondria and thus increased priming. These changes in Bax subcellular distribution can occur rapidly, indicating that changes in mitochondrial priming can adapt quickly to changing conditions. This provides cells with the ability to fine-tune their response to apoptotic stresses based on varying intra and extracellular conditions.

Dynamic regulation of priming during mitosis

Well-established links exist between Bcl-2 proteins and cell cycle progression (Zinkel et al., 2006). Actively

cycling cells are more sensitive to apoptosis, whereas cells in G0 are more resistant. Furthermore, driving cells into cell cycle, for example, by expressing oncogenic Myc, induces a pro-apoptotic state (Evan and Littlewood, 1998; Juin et al., 2002). Conversely, overexpression of Bcl-2 can be anti-proliferative as well as anti-apoptotic (Vairo et al., 2000). Such observations indicate reciprocity between proliferation and apoptosis. Indeed, as Myc drives cell cycle progression (Daksis et al., 1994; Vlach et al., 1996; Pérez-Roger et al., 1997), it also alters the landscape of apoptotic gene expression to increase mitochondrial priming, up-regulating the BH3-only proteins Bid, Bim and NOXA and down-regulating anti-apoptotic Bcl-XL (Conacci-Sorrell et al., 2014). Conversely, inhibiting Myc reduces mitosis-associated priming (Topham et al., 2015). The outcome is to ensure that failure in correct chromosomal segregation activates mitochondrial apoptosis that is dependent upon Bax and Bak. This apoptotic response to a failure to correctly exit mitosis is the rationale behind those chemotherapeutic agents collectively termed anti-mitotics (Dumontet and Jordan, 2010). These drugs, such as taxanes and vinca alkaloids, are widely used to treat a range of cancers, including breast, lung and ovarian.

As with other apoptotic stimuli, cells respond with considerable heterogeneity to delayed mitotic exit (Gascoigne and Taylor, 2008). A mitotic cell will not enter anaphase until its chromosomes have correctly aligned and attached to spindle microtubules, a requirement for satisfying the spindle assembly checkpoint (SAC). SAC inactivation following correct kinetochore attachment allows anaphase-promoting complex/cyclosome (APC/C) to ubiquitinate cyclin B, promoting its rapid degradation to initiate mitotic exit. The preferred response for a cell that fails to satisfy the SAC is apoptosis (Topham and Taylor, 2013). However, there is a gradual degradation of cyclin B during mitosis whether or not the SAC is active, meaning that if the cell does not die it will eventually undergo 'slippage', exiting mitosis without dividing. Following slippage, cells may senesce, die or enter further cycles of proliferation, leading to aneuploidy (Rieder and Maiato, 2004). A population of cells treated with an anti-mitotic show all these possible outcomes. Not only that, but the cells that do die in mitosis, do so after a wide range of different times. These observations led to the competing networks model, which proposes that upon entering mitosis, a cell initiates two independent timers (Huang et al., 2010; Topham and Taylor, 2013). One, driven by cyclin B degradation, will result in slippage. The second is an accumulation of a pro-apoptotic signal that will end in MOMP. Whichever timer breaches its threshold first determines the outcome. How the apoptotic timer is controlled is not completely clear, but Mcl-1 is a major component.

Mcl-1 is a unique anti-apoptotic Bcl-2 protein, with a large N-terminal domain distinct from Bcl-2, Bcl-XL, Bcl-W or Bcl-A1 (Thomas et al., 2010). This N-terminus contains multiple regulatory sites, including proline, glutamic acid, serine and threonine (PEST) sequences, sites of ubiquitination, phosphorylation and proteolysis. Mcl-1 has a particularly short half-life, due to proteasomal degradation. This instability of Mcl-1, compared to other multi-domain Bcl-2 proteins, is exploited in mitotic cells to allow the loss of its anti-apoptotic activity over time (Harley et al., 2010; Wertz et al., 2011; Sloss et al., 2016). However, the mechanism controlling Mcl-1 degradation during mitosis is not as clear as that for cyclin B. A number of protein kinases have been shown to phosphorylate Mcl-1, promoting its ubiquitination, including Jnk, p38 and Cdk-1 (Thomas et al., 2010). Furthermore, at least three E3-ligases have been implicated in its degradation in mitosis. The E3 ligase Mcl-1 ubiquitin ligase E3 (MULE) was shown to be important in Mcl-1 stability (Zhong et al., 2005), CDK1-mediated phosphorylation of Mcl-1 was shown to promote its degradation mediated by APC/C (Harley et al., 2010) and FBW-7/SCF, which targets Mcl-1 for ubiquitin-mediated degradation in interphase, was also shown to play a significant role in mitotic cells (Inuzuka et al., 2011; Wertz et al., 2011). However, a recent study has suggested that in colon carcinoma cell lines, none of these mechanisms has a dominant effect on promoting Mcl-1 loss during mitotic arrest (Sloss et al., 2016). Despite this lack of consensus as to the mechanism, it is clear that stabilisation of Mcl-1 in mitosis has a significant impact in delaying onset of DiM (Tunquist et al., 2010). In a recent study, inhibiting the loss of Mcl-1 in RKO cells that were blocked in mitosis with a degradation-resistant Cdc-20 resulted in a significant increase in time to DiM (Sloss et al., 2016). If Mcl-1 was knocked down under the same conditions, there was a striking synchronisation of apoptosis as cells entered mitosis, an effect not observed if Bcl-XL was depleted instead.

It is significant that Mcl-1 knockdown did not increase apoptosis prior to mitosis (Sloss et al., 2016). Thus, entry to mitosis results in a marked increase in apoptotic priming that makes cells dependent upon Mcl-1, and thus sensitive to its loss. This apoptotic priming upon mitotic entry can be demonstrated functionally. For example, DLD-1 colon cancer cells, normally resistant to DiM, tend to undergo slippage and become sensitised to BH3 mimetics by taxol treatment (Wang et al., 2014). Thus, regulation of the pro-apoptotic Bcl-2 proteins must also be a key

part of regulating the DiM timer. To address this, siRNA screens for factors that favour DiM have been performed (Diaz-Martinez et al., 2014; Topham et al., 2015). Although the specific details vary, the results show that cells reposition their Bcl-2 expression profile prior to mitotic entry. One study found Myc to be a key determinant for colon carcinoma cells to undergo DiM (Topham et al., 2015). Myc reprogrammed the apoptotic landscape prior to mitosis, downregulating Bcl-XL, and upregulating the BH3-only proteins Bim, Bid and NOXA. If Myc expression was reduced, this reprogramming was not as evident and cells became prone to slippage. A similar siRNA screen in HeLa cells did not identify Myc, but did find NOXA as a key determinant promoting DiM (Diaz-Martinez et al., 2014). NOXA is interesting as it has a binding specificity for Mcl-1 (Chen et al., 2005). As there is significant interline variation in the response to delayed mitotic exit, it is not surprising that studies in different cells find differences in the specific proteins implicated. However, a common theme is the realignment of pro- and anti-apoptotic Bcl-2 proteins, indicating that mitochondrial priming is a significant determinant for the correct response to failure to exit mitosis.

Altering the profile of Bcl-2 expression prior to mitosis only partly explains the response during mitosis. The initiation of MOMP is the critical point, and this must occur in the mitotic cell. As cells in mitosis are transcriptionally inactive, variations in Bcl-2 proteins during this period cannot be explained by transcriptional regulation mediated by Myc, although there is evidence that some protein translation of Mcl-1 may still occur (Sloss et al., 2016). During mitosis, a significant amount of the cells' proteome undergoes mitosis-specific phosphorylation (Dephoure et al., 2008), and it is clear that post-translational regulation of Bcl-2 proteins plays an important role apoptosis regulation at this time. A number of Bcl-2 family proteins are subject to phosphorylation in mitosis. Mcl-1 has been discussed above, where several phosphorylation sites have been shown to promote its ubiquitiation. Phosphorylation of other Bcl-2 proteins may also shift either the state of priming or the contribution of specific proteins for this priming. Phosphorylation of Mcl-1 on serine 64 in mitosis may increase its anti-apoptotic function, separate to its degradation (Kobayashi et al., 2007; Harley et al., 2010). At the same time, Bcl-2 and Bcl-XL also become phosphorylated, reducing their anti-apoptotic activity (Basu and Haldar, 2003; Terrano et al., 2010). Bcl-XL phosphorylation on serine 62 in mitosis has been shown to reduce its ability to supress Bax, thus increasing the priming of MDA-MB-231 breast cancer cells when treated with paclitaxel (Bah et al., 2014).

The pro-apoptotic BH3 proteins are critical to priming, and a number of these are post-translationally regulated in mitosis. The longest splice variant of Bim, Bim-EL, is phosphorylated on a number of sites by several kinases, including Erk and Rsk (Lei and Davis, 2003; Putcha et al., 2003; Ewings et al., 2007). Bim is phosphorylated in mitosis by Aurora A, regulating binding to the E3 ligase SCF-βTrCP1 (Moustafa-Kamal et al., 2013). This results in ubiquitination and degradation of Bim-EL, which would appear to contradict the observation that cells become primed in mitosis. However, it is the total BH3 profile that ultimately determines this sensitivity, and Bid and Bad are also phosphorylated in mitosis (serine 67 and 128, respectively), which increases apoptotic sensitivity (Konishi et al., 2002; Berndtsson et al., 2005; Mac Fhearraigh and Mc Gee, 2011; Wang et al., 2014). In particular, Bid has recently been shown to be phosphorylated on serine 67 (serine 66 in mouse), and this is required for the increase in priming seen in colon cancer cells, and for the RKO cells, in particular, to undergo DiM (Wang et al., 2014). Bid phosphorylation is lost at anaphase, suggesting that the temporal control achieved by such mechanisms allows cells to reduce priming when the SAC is satisfied and the cell proceeds into anaphase.

A picture thus develops whereby as cells approach mitosis, they reposition their Bcl-2 expression profile in anticipation of switching their priming during division (Figure 3). Upon entry to mitosis, a pattern of post-translational modifications on both BH3-only and multi-domain Bcl-2 proteins then provide temporal precision in this regulation. Finally, as the conditions for mitotic exit are met, priming can be rapidly reset.

Coupling between the apoptosis timer and mitotic exit

Bcl-2 proteins are not just limited to acting as 'end-point regulators' controlling MOMP during the cell cycle. Bcl-2 proteins also act upstream of MOMP to influence entry and progression through the cycle (Vairo et al., 1996; Zinkel et al., 2006). High levels of Bcl-2 or Bcl-XL slow the entry of cells into the cell cycle from G0 and their progression to S-phase. While the exact mechanism by which this is achieved still remains unclear, it appears that overexpression of Bcl-2 leads to the elevation levels of the retinoblastoma (RB)-related protein p130, causing the repression of E2F and the transcription of G1 associated genes. In addition, Bcl-2 and Bcl-XL overexpression inhibits the degradation of the G1 cyclin-dependent kinase inhibitor

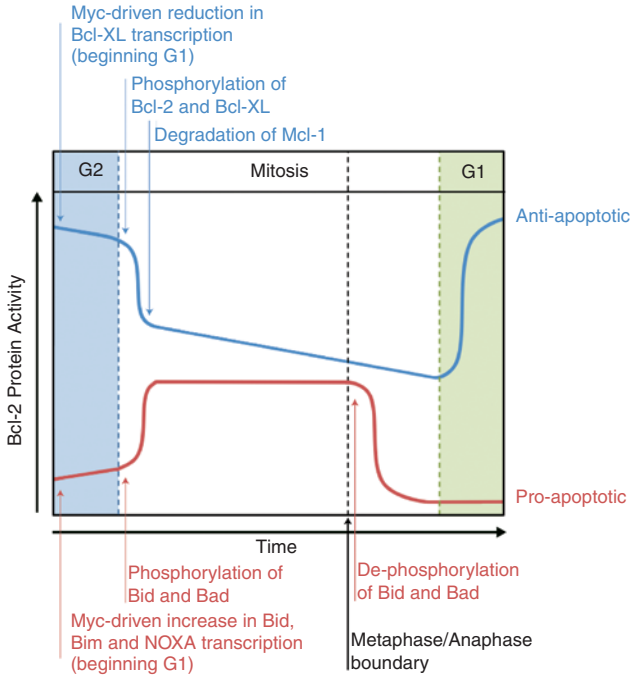

Figure 3: Fluctuations in Bcl-2 protein activity during mitosis. As the cell commits to entering the cell cycle, Myc alters the landscape of apoptotic gene expression, upregulating the pro-apoptotic BH3-only proteins Bid, Bim and NOXA, while downregulating the anti-apoptotic protein Bcl-XL. On entry to mitosis, both pro- (Bid and Bad) and anti-apoptotic proteins (Bcl-2 and Bcl-XL) become phosphorylated. The phosphorylation of Bid and Bad increases their pro-apoptotic activity, while the phosphorylation of Bcl-2 and Bcl-XL reduces their ability to protect against MOMP. The combination of these phosphorylation events increases priming specifically in response to mitotic entry. At the same time, Mcl-1 is degraded, progressively diminishing the cells' ability to prevent the induction of apoptosis. On satisfaction of the SAC and the transition from metaphase to anaphase, the phosphorylation of Bid, Bim, Bcl-XL and Bcl-2 is lost and mitochondrial priming resets to baseline levels.

p27 (O'Reilly et al., 1996; Vairo et al., 2000; Greider et al., 2002). Mcl-1 overexpression also retards cycle progression, as Mcl-1 forms small proteolytic fragments which interact with CDK1 and promote the phosphorylation of DNA damage checkpoint kinase 1 (CHK1), arresting the cell at the DNA damage checkpoints even in the absence of DNA damage (Fujise et al., 2000; Jamil et al., 2005, 2008). Conversely, the pro-apoptotic Bcl-2 proteins Bax and Bad promote progression through the cell cycle, increasing the G1 to S-phase transition, and can allow cells to bypass arrest at the restriction point due to serum starvation, high confluence environments and Bcl-2 overexpression (Borner, 1996; O'Reilly et al., 1996; Chattopadhyay et al., 2001; Maslyar et al., 2001; Janumyan et al., 2003).

Similarly in mitosis, the two competing networks controlling slippage and MOMP may not be entirely independent of each other (Fava and Villunger, 2014). Indeed, manipulating apoptotic regulators upstream of MOMP not only delayed or prevented DiM, it also altered the timing of slippage. In U2OS cells, which are prone to slippage (as opposed to HeLa or RKO cells, both of which are biased toward DiM), depletion of Bax and Bak not only reduced the total amount of cell death seen in taxol-mediated arrest, but surprisingly also increased the time cells remained arrested before slippage (Diaz-Martinez et al., 2014). This effect was not due to anything downstream of MOMP, and appeared to be connected to the ability of Bax and Bak to regulate mitochondrial dynamics. Bax- and Bak-deficient cells have been shown to have hyper-fragmented mitochondria (Karbowski et al., 2006). Bax localises to sites of mitochondrial fission during apoptosis, along with Drp1 and Mfn2, which promote mitochondrial fission and fusion, respectively (Karbowski et al., 2002). Interestingly, knock down or inhibition of Drp1 had the same effect as loss of Bax and Bak on the extension of time to slippage in U2OS cells, suggesting that slippage may be regulated by the effects on mitochondrial function (Diaz-Martinez et al., 2014). How this occurs is unclear, but one possibility may be that it is linked to the role of mitochondrial dynamics in their segregation during mitosis.

Similarly, altering the slippage timer was found to impact on the apoptotic timer in arrested cells. The same study found that depletion of the SAC inhibitor $p31^{comet}$ increased the time U2OS cells remained arrested in taxol before undergoing slippage (Diaz-Martinez et al., 2014). However, depletion of $p31^{comet}$ was also found to decrease the time arrested cells took until undergoing DiM. $p31^{comet}$ inhibits the SAC component MAD2, which determines activity of APC/C to promote the transition to anaphase (Varetti et al., 2011). Depletion of $p31^{comet}$ thus enforced inhibition of APC/C. The authors found that the effect of $p31^{comet}$ loss on the reduced time to DiM was not simply due to altered dynamics of Mcl-1 degradation in the arrested cells (Diaz-Martinez et al., 2014). In another study, overexpression of Mcl-1 increased the time arrested colon cancer cells took to undergo slippage as well as affecting time to DiM (Sloss et al., 2016). Conversely, Mcl-1 inhibition accelerated the time to slippage in the slippage-prone DLD1 cells. As Mcl-1 may be a substrate for APC/C, the authors suggested that the presence of a potential binding motif for this E3 ligase may result in competition with APC/C for binding to other substrates, including cyclin B, resulting in delayed slippage. This interpretation would suggest that the two networks tend to interfere with each other. Either way, the emerging data indicate that the response of cells to anti-mitotic drugs is complex, and directly targeting either the SAC or the apoptotic regulators may

have consequences in how the other networks respond, whether by direct or indirect means.

The increased understanding of how cells respond to delayed exit from mitosis is providing much needed detail on the mechanisms by which commonly used chemotherapeutic agents may work. Understanding these processes will help devise strategies whereby cancers cells will undergo the preferred response of dying when exposed to these treatments.

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