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Commentary Open Access

Targeting Apoptosis Signalling in Cancer: How Decreasing BAK Phosphorylation *via* BMX Inhibition Increases Sensitivity to Cytotoxic Drugs

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Commentary

At the molecular level there exists a fine balance between death and survival, which is essential in healthy cells for normal foetal development and for the clearance of damaged cells. However, more often than not, this balance is disrupted in diseases resulting in either too much cell death, for example in neurodegenerative disorders, or insufficient cell death as observed in cancer. Cancers are genetically heterogeneous and evolve during tumourigenesis, a process that helps cancer cells survive, by evading cell death, promoting proliferation and activating migration and invasion [1]. Targeting apoptosis, particularly for the treatment of cancer, has therefore become an increasingly attractive intervention strategy. Drugs approved for clinical use as well as many in the development pipeline aim to alter apoptotic signalling outcome or to trigger initiation of apoptosis, for example SMAC mimetics or BH3 mimetic Venetoclax respectively. However, these approaches have not been without their complications with both on-target and off-target dose limiting toxicity reported, as well as the development of drug-induced resistance (reviewed in [2]). Despite the exciting progress that has been made in understanding and targeting the complex pathways that commits a cell to die by apoptosis, it is becoming increasingly clear that simply characterising the effector pathways is not enough [2]. Understanding the regulatory pathways and checkpoints at the crucial commitment points that trigger these executioner cell death pathways is equally important and could be the key to enable successful modulation of death pathways that determine cell fate.

Intrinsic or mitochondrial apoptosis depends on activation of the BCL-2 effector proteins BAK and/or BAX which, once activated, are able to form first dimers then higher order multimers which disrupts the outer mitochondrial membrane [3]. This loss of membrane integrity results in the release of apoptogenic factors such as cytochrome c which is required to form an active apoptosome complex and activates downstream caspases. Recent studies, however, have found that it is activation of BAK/BAX resulting in the loss of mitochondrial outer membrane potential (MOMP) as the irreversible commitment step in the process. Therefore, BAK/BAX exert their effects at a crucial point in the apoptotic cascade and regulation of their activation status is critical in determining cell fate. BAK is constitutively present in the outer mitochondrial membrane, and requires tight regulation to ensure that aberrant induction of apoptosis does not occur. Two key sequential dephosphorylation events serve to regulate the ability of BAK to undergo activation in response to death signals. The initial BAK dephosphorylation at Y108 by PTPN5 [4] is the key switch that enables BAK activation to proceed, followed by dephosphorylation of S116 by PP2A [5]. Once these two phosphorylation marks have been removed then BAK activation can proceed [3].

In our recent article "BMX Negatively Regulates BAK Function Thereby Increasing Apoptotic Resistance to Chemotherapeutic Drugs" we reported BMX as the first kinase to be identified which, in concert with PTPN21, directly phosphorylates the key Y108 regulatory residue on the apoptotic effector protein BAK [6]. Phosphorylation at residue Y108 maintains BAK in an inactive conformation preventing initiation the intrinsic apoptosis pathway and preventing BAK activation. This increases the apoptotic threshold of the cell making the cell more resistant the cell killing. Overexpression of BMX in our cell line models maintained BAK Y108 phoshorylation and caused the cells to become resistant to the chemotherapeutic agents tested. Importantly, BMX has been found to be up-regulated in numerous cancer types including breast, prostate and colon, suggesting that during the 'evolution' of the cancer, elevated levels of BMX helps cancer cells survive.

BMX is known to have multiple downstream target proteins including STAT3, AKT and PAK in addition to BAK, but also integrates signalling pathways from multiple diverse networks, including PI3K, SRC and FAK. Taken together, the function of BMX a key regulator of proliferation and migration, and anti-apoptotic activity, pinpoints BMX as a therapeutic target of major interest as it can respond to multiple stimuli, including those generated by diverse chemotherapeutic agents. There currently exists a strong commercial interest in developing BMX inhibitors (reviewed in [7]), and interestingly, existing non-reversible EGFR inhibitors have also been found to inhibit BMX activity. It is noteworthy that despite the reported roles of BMX in proliferation and inhibition of apoptosis, homozygous BMX knockout mice do not have any obvious phenotype [8] and have a normal lifespan, which suggests that although BMX is a key regulatory protein it is not an effector. These findings agree with our published observations, that knocking down BMX using RNAi although able to both reduce the basal levels of BAK Y108 phosphorylation was by itself not sufficient to trigger apoptosis. From a therapeutic viewpoint this could be of benefit as BMX inhibition may potentially have limited toxicity. In our study, it was the combination of BMX knockdown with existing chemotherapeutic agents that had the most profound affect. We found that doses of drugs that themselves induced little or no cell death in BMX-expressing cells, were able to induce extensive cell death when BMX levels were knocked down with RNAi. Others have also reported modulation of BMX activity sensitizes cells to therapeutic agents. Investigation of the effects of combination of a dual PI3K/mTOR inhibitor which modulated BMX activity with the pro-apoptotic BH3 mimetic ABT737, revealed an increase in cellular sensitivity with the combination compared to either agent alone. As shown in (Figure 1) and outlined above PI3K is one of the upstream regulators of BMX, and interestingly it was found that it was the effect of PI3K inhibition on BMX activity rather than on AKT or mTOR that was responsible for the increased sensitivity observed both in colorectal cancer cell models [9] and small cell lung cancer

[10]. Together with our mechanistic study these findings suggest strongly that when it comes to BMX inhibition, either directly [6] or *via* modulating upstream regulators such as PI3K [9,10], a combinatorial approach would be most effective. However, it remains to be established whether upstream PI3K inhibition is able to modulate BMX activity with enough amplitude and duration to be effective or whether directly targeting BMX may be a better approach.

Cancer stem cells have been described for many tissues, and their longevity and resistance to the effects of standard chemotherapy and radiotherapy infers that they can accumulate more cellular mutations than shorter-lived more proliferative cells. These characteristics have profound implications for disease initiation, progression and approaches to treatment [11-15].

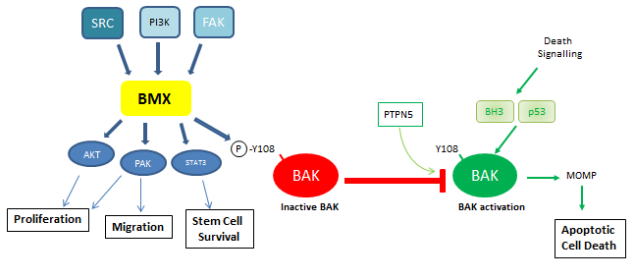

Figure 1: Schematic diagram for BMX regulation. Upregulation of BMX activity results in phosphorylation of its downstream substrates including AKT, PAK, STAT3 and BAK resulting in increased proliferation and migration and inhibition of the induction of apoptosis.

A further potential advantage to targeting BMX therapeutically is that BMX is required for stem cell maintenance and survival [16]. Therefore, up-regulation of BMX gives a survival benefit both to primary tumours and the cancer stem cells, which are highly resistance to apoptosis and survive many chemotherapeutic treatments. Targeting BMX in this setting, therefore, might have a more profound effect by killing both proliferating cells in the primary tumour and eliminating the stem cell fraction that drives tumour growth and recurrence. However, in both cases it is important to remember that inhibition of BMX alone will not induce cell killing, it will lower the cellular apoptotic threshold, by making more BAK in an 'activation competent' state and therefore making the cells more vulnerable to killing induced by other agents. The ability to determine the mitochondrial apoptotic threshold *via* BH3 profiling has been extensively studied [17,18], however to date this only gives an indication of cancers that will be sensitive to a given treatment. This technique however could be extended to determine whether successful modulation of the apoptotic threshold has been achieved.

It is clear that apoptotic signalling pathways and the proteins that determine the outcome of these pathways are tightly regulated to maintain the cellular balance between life and death. Interestingly, many of the key regulatory proteins are involved in multiple signalling pathways, the balance of which determines the overall cell fate. During cancer evolution proteins that provide a survival benefit are often up-regulated, and alter the 'normal' balance within the cell, usually shifting this balance in favour of survival and increasing the apoptotic threshold. The detailed mechanistic insights into how BMX regulates BAK activation and cell killing we have uncovered provide an opportunity to apply an interventional therapeutic strategy that lowers the cellular apoptotic threshold and hence the amount of drug needed to be effective in cell killing. In light of this a paradigm shift is required where target modulation must guide the way in which drug combinations are trialled, no longer focusing only on the maximum tolerated dose, but instead on how to best augment cell killing. Such an approach can deliver huge potential benefits for patients by reducing unwanted adverse effects to promote tolerability, while maintaining the effectiveness of harsh chemotherapeutic regimens.

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