The Tangled Circuitry of Metabolism and Apoptosis

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For single-cell organisms, nutrient uptake and metabolism are central to the fundamental decision of whether to grow or divide. In metazoans, cell fate decisions are more complex: organismal homeostasis must be strictly maintained by balancing cell proliferation and death. Despite this increased complexity, cell fate within multicellular organisms is also influenced by metabolism; recent studies, triggered in part by an interest in tumor metabolism, are beginning to illuminate the mechanisms through which proliferation, death, and metabolism are intertwined. In particular, work on Bcl-2 family proteins suggests that the signaling pathways governing metabolism and apoptosis are inextricably linked. Here we review the crosstalk between these pathways, emphasizing recent work that illustrates the emerging dual nature of several core apoptotic proteins in regulating both metabolism and cell death.

Introduction
Eukaryotic metabolism encompasses a remarkably complex and tangled circuitry of catabolic and anabolic pathways. These pathways yield countless metabolic intermediates, many of which have potential signaling functions. Although our understanding of how metabolic pathways and metabolites integrate with cell fate signaling is still in its infancy, the interplay between metabolism and cell death has garnered new interest due to its emerging role in some of the most devastating human diseases: cancer, Alzheimer’s disease, and diabetes. Here we discuss how apoptotic signaling is linked to several metabolic pathways, including glycolysis, the tricarboxylic acid cycle (TCA cycle), and the pentose phosphate pathway (PPP) (summarized in Figure 1).

The Fate of Glucose
Glucose, a key source of both metabolic fuel and new cell mass, is taken up by the cell in a regulated manner via glucose transporters (GLUTs) (reviewed in Thorens and Mueckler, 2010). Once inside the cell, glucose is phosphorylated to glucose-6-phosphate (G6P) by hexokinases, at which point G6P can either continue through glycolysis to generate pyruvate for the TCA cycle, or be shunted into the PPP to generate ribose-5-phosphate and NADPH. PPP-mediated production of NADPH protects cells from oxidative damage by generating reduced glutathione, which neutralizes hydroperoxides. NADPH is also an essential cofactor for the reductive biosynthesis of nucleotides, amino acids, and fatty acids. In addition, PPP-generated ribose-5-phosphate is further metabolized through the nonoxidative arm of the PPP and is ultimately used for the synthesis of nucleic acids. In this regard, the biosynthetic capacity of a cell is highly dependent on the PPP. As such, the upregulation of PPP activity is a hallmark of rapidly proliferating cells like those found in tumors or embryonic tissues.

The glycolytic end product, pyruvate, enters the mitochondria and undergoes a series of oxidative reactions via the TCA cycle. Pyruvate processing within the TCA cycle ultimately yields two ATP and six NADH molecules per molecule of glucose. TCA cycle-generated NADH is then used as a reducing agent in oxidative phosphorylation to generate a high yield of ATP. In addition to glucose, the TCA cycle also receives input from cytosolic amino acids that are transported into the mitochondria to generate TCA cycle intermediates (a-ketoglutarate, succinyl-CoA, fumarate, oxaloacetate, and acetyl-CoA). For example, a key energy source for tumor cells, glutamine, is converted to glutamate in the cytosol by glutaminase. Glutamate is then transported into the mitochondria, where it is converted to α-ketoglutarate. One product of the TCA cycle particularly relevant to this review is acetyl-CoA. Citrate, which is transported out of the mitochondria into the cytosol, is converted to oxaloacetate and acetyl-CoA by ATP citrate lyase (ACL). Once cytosolic, acetyl-CoA is converted to malonyl-CoA, which is used as a building block for fatty acid biosynthesis (summarized in Figure 1). Citrate-derived acetyl-CoA is also used as the primary source of the acetyl group in lysine acetylation and N-alpha acetylation (to be discussed later).

Mitochondria: The Nexus of Metabolic and Apoptotic Signaling
Numerous studies over the past 10–20 years have revealed a complex picture of mitochondria—far removed from the traditional textbook image of morphologically static ATP generators. For one, mitochondria are the focal point of apoptotic signaling. Death stimuli ranging from DNA damage to metabolic stress to immune cell-mediated death receptor ligation converge on the mitochondria to trigger the release of cytochrome c from the mitochondrial intermembrane space. The release of cytochrome c (generally considered a critical decision point in apoptosis [Llambi et al., 2011]) occurs through a process known as mitochondrial outer membrane permeabilization (MOMP), which involves activation of the Bcl-2 family members Bax and Bak at the surface of mitochondria. Once in the cytosol, cytochrome c triggers the oligomerization of apaf-1 with the cysteine-aspartic protease caspase-9 to form the apoptosome, which goes on to activate the executioner caspases, caspase-3 and caspase-7. These terminal caspases cleave various cellular substrates and...
ultimately dismantle the cells in the tidy manner characteristic of apoptosis (summarized in Figure 1).

Mitochondria are also highly dynamic organelles, continuously rearranging themselves through fission and fusion (reviewed in Martinou and Youle, 2011). Notably, the Bcl-2 family proteins, long regarded as apoptotic regulators, have been shown to affect rates of mitochondrial fission and fusion. While their precise functions in controlling cell viability are still hotly debated, fission and fusion have been shown in some systems to impact both metabolism and apoptosis (Martinou and Youle, 2011). Taken together, the fact that many of the cell's key metabolic and cell fate signaling pathways are routed through mitochondria suggests that mitochondria are a likely site of crosstalk between these pathways.

To date, probably the most direct example of a metabolism-apoptosis link lies with the dual functionality of cytochrome c. Cytochrome c is a heme protein associated with the inner mitochondrial membrane (IMM). Its long-known metabolic role is to pass an electron from respiratory complex III to complex IV in order to promote ATP generation through oxidative phosphorylation. In 1996, through a series of elegant biochemical experiments published in their seminal Cell paper, Xiaodong Wang and colleagues found that cytochrome c was necessary for activation of caspase activity (Liu et al., 1996). While the factors that control mitochondrial cytochrome c release (i.e., Bax/Bak and other Bcl-2 family proteins—to be discussed later) seem to be a primary locus of regulation, the apoptotic activity of cytochrome c itself is also affected by glucose metabolism.

A common feature of neurons, like cancer cells, is their heavy use of glucose as a metabolic fuel. In addition, both neurons and cancer cells are refractory to cytochrome c-induced caspase activation, even when cytochrome c is directly microinjected into the cytosol (Schafer et al., 2006; Vaughn and Deshmukh, 2008). Vaughn and Deshmukh linked these observations by showing that glucose-stimulated production of intracellular glutathione, as a result of NADPH production through the PPP, led to inactivation of cytochrome c by keeping it in its reduced state (Vaughn and Deshmukh, 2008). As they further showed, induction of apoptosis led to an increase in reactive oxygen species (ROS) that subsequently triggered oxidation of cytochrome c, thereby rendering it capable of caspase activation. This study and others (Brown and Borutaite, 2008; Li et al., 2008) affirmed that oxidation of cytochrome c is indeed important for apoptosis. An implication of these studies is that the native, steady-state redox status of different tissues/cells will affect their sensitivity to cytochrome c. As NADPH levels are both predominately controlled by the PPP and a key determinant of cellular redox status due to the role of NADPH in GSH production, these studies suggest that cells with high PPP activity will be resistant to caspase activation downstream of MOMP. In support of this idea, in tumor cells with high endogenous PPP activity, inhibition of the PPP by dihydroepiandrosterone (DHEA) is sufficient to generate the oxidative cytosolic environment necessary to sensitize cells to caspase activation induced by microinjected cytochrome c (Vaughn and Deshmukh, 2008).
The Dual Nature of Bcl-2 Family Proteins

Proapoptotic Bcl-2 Proteins

Bcl-2 family proteins are the chief controllers of MOMP in the cell. The Bcl-2 family is divided into pro- and antiapoptotic members, but all share combinations of α-helical regions, known as Bcl-2 homology (BH) domains. These domains largely determine each Bcl-2 member’s function in the apoptotic cascade. The proapoptotic Bcl-2 family proteins (e.g., Bax, Bak, Bid, Bim, Bad, Puma, and Noxa) all contain a single amphipathic BH segment, known as the BH3 domain. Bax and Bak, which are the gateway controllers of MOMP, possess multiple BH domains, while the rest of the proapoptotic family members (e.g., Bad, Bim, Bim, Puma, and Noxa) only possess the BH3 domain (hence their common name, “BH3-only proteins”). The precise mechanism by which the BH3-only proteins promote apoptosis can differ. Some, like Bid and Bim, are thought to interact with and promote the activation of Bax and Bak directly. In contrast, Bad, Puma, and Noxa can act as “derepressors,” neutralizing the antiapoptotic activity of Bcl-xL and Bcl-2 to facilitate Bax/Bak activation.

While Bax is traditionally considered a tumor suppressor due to its role in triggering MOMP, early studies of Bax-deficient mice revealed the presence of both hyperplasia and hypoplasia depending on tissue type (Knudson et al., 1995). This study and others have provided hints that Bax may have functions unrelated to MOMP. For one, Bax has been shown to regulate the levels of ROS in healthy neurons (Kirkland and Franklin, 2007). Bax and Bak together also regulate ER calcium homeostasis in lymphocytes (Jones et al., 2007). In addition, Bax has been observed to form foci at mitochondrial fission sites in healthy cells (Karbowski et al., 2002), and deletion of Bax and Bak alters the normal rate of mitochondrial fusion (Karbowski et al., 2006). The impact of Bax on mitochondrial fission/fusion in particular may, by extension, impact mitochondrial metabolism (Zorzano et al., 2010). In this regard, a recent study by Boohaker et al. (2011) showed that the Bax-deficient cells display decreased oxygen consumption/ATP levels and increased glycolysis, all of which could be rescued by Bax overexpression. Importantly, the rescue of these metabolic defects was observed with a Bax mutant lacking the C-terminal helix required for MOMP, suggesting that the metabolic and apoptotic functions of Bax are mechanistically distinct (Boohaker et al., 2011). However, whether this metabolic function of Bax is related to its effect on mitochondrial dynamics is still unclear.

Bax activation during MOMP has also been mechanistically linked to the metabolism of ceramide, a lipid metabolite long suspected to play a role in apoptosis. Ceramide is upregulated by numerous cellular stresses. Early cell-free experiments (in Xenopus egg extract, for example) implicated ceramide in the activation of the apoptotic cascade (reviewed in Woodcock, 2006). Ceramide has even been proposed to induce MOMP directly by forming pores in the outer mitochondrial membrane (OMM) (Colombini, 2010). Ceramide can be produced through multiple mechanisms: de novo through the combination of serine and palmitoyl-CoA (products of glycolysis and fatty acid synthesis, respectively), through salvage pathways, or by sphingomyelinase (SMase)-mediated hydrolysis of the common membrane phospholipid sphingomyelin (also a product of de novo ceramide synthesis). A role for the latter mechanism in promoting Bax/Bak-mediated MOMP has been reported (Chipuk et al., 2012). Specifically, Chipuk and colleagues found that an SMase both cofractionated with mitochondrial-associated membranes (MAMs) and generated a mitochondrial pool of ceramide. This pool of ceramide can be broken down into sphingosine-1-phosphate (S1P) and the fatty aldehyde hexadecenal (hex). The authors found that hex interacts directly with Bax to facilitate Bax activation in response to truncated Bid (tBid), while S1P seems to promote Bak activation (Chipuk et al., 2012). Although little is known about the cellular activates of hex, S1P has numerous cell signaling functions. In apparent contrast to the mechanism described above, S1P is commonly prosurvival counterbalanced to the proapoptotic activity of ceramide (reviewed in Van Broocklyn and Williams, 2012), possibly due to its role in the activation of ERK survival kinase signaling (Cuvillier et al., 1996). Resolving these apparently disparate functions will likely require a better understanding of S1P’s mitochondrial function. Indeed, despite its numerous cytosolic signaling roles, our understanding of S1P’s mitochondrial function is relatively limited (Strub et al., 2011). Further studies will be required to parse the mechanistic details of hex- and S1P-mediated Bax/Bak activation and to understand how this mechanism fits into the larger metabolic picture.

A link between nutrient pathways and the BH3-only protein Bad was first observed in the 1990s and has since been corroborated and extended by multiple groups (Datta et al., 2002; del Peso et al., 1997; Zha et al., 1996). In 1996, the Korsmeyer group found that supplementation of cells with the survival factor interleukin-3 (IL-3), which promotes glucose uptake and metabolism, induced the phosphorylation of Bad at S112 and S136 (mouse Bad numbering). This phosphorylation consequently led to an interaction between Bad and 14-3-3, resulting in the suppression of Bad’s proapoptotic activity (Zha et al., 1996). Later, Dandial et al. found that a pool of Bad resides at the mitochondria in a multiprotein complex with glucokinase (GK) (Danial et al., 2003), a hexokinase whose expression is limited to β-islet cells, hypothalamic neurons, and hepatocytes. By showing that Bad serves as a scaffold for this complex and is required for GK activity and normal glycolytic function, Dandial and colleagues implicated Bad as a key regulator of glucose metabolism (Danial et al., 2003). Toggling the function of Bad between metabolism and apoptosis, and working in concert with the phosphorylations at S112 and S136, is a third phosphorylation site at S155 (within the BH3 domain). When S155 is phosphorylated, Bad loses its ability to antagonize the antiapoptotic Bcl-xL and begins to promote GK-mediated glucose metabolism (Figure 2) (Danial, 2008; Datta et al., 2000; Tan et al., 2000). Conversely, dephosphorylation at all three sites increases apoptotic sensitivity by lowering the threshold for MOMP (Danial, 2008). Together, these studies help to set the paradigm for how Bcl-2 family proteins might coordinately regulate apoptosis and metabolism.

While no other proapoptotic Bcl-2 family protein to date has been tied to metabolism with the same mechanistic detail as Bad, others are clearly responsive to metabolism. The
BH3-only protein Puma, which can bind and inhibit all antiapoptotic Bcl-2 family members as well as induce Bax activation directly, is modulated by glycolytic activity (Zhao et al., 2007). In a hematopoietic precursor cell model, IL-3 withdrawal causes a reduction in glycolysis and an increase in Puma expression. Using this system, Zhao et al. demonstrated that maintaining glycolytic activity (by forced expression of Glut1 and hexokinase) after IL-3 withdrawal was sufficient to attenuate Puma induction and block cell death (Zhao et al., 2007). Additionally, glucose deprivation, even in the presence of IL-3, induced Puma expression and Puma-dependent cell death; therefore, a reduction in glycolysis is likely a key apoptotic stimulus in response to IL-3 withdrawal (Zhao et al., 2007). In this system, the link between metabolic stress and Puma was traced to p53. p53 is suppressed by high glycolytic activity and activated by IL-3 withdrawal through a protein kinase C-dependent pathway, which leads to the transcriptional upregulation of Puma (Mason and Rathmell, 2011; Zhao et al., 2007).

In addition to Puma, the BH3-only protein Noxa has also been linked to glucose deprivation-induced apoptosis. TCR-mediated activation of T cells leads to a PKC-dependent (and p53-independent) induction of Noxa (Alves et al., 2006). Induction of Noxa sensitized cells specifically to nutrient deprivation but failed to sensitize against other stresses (e.g., DNA damage, ER stress, oxidative stress). Additionally, RNAi-mediated depletion of Noxa rendered leukemic and proliferating primary T cells resistant to apoptosis induced by glucose-limiting conditions. Interestingly, in addition to TCR engagement, Noxa upregulation was also observed in response to other mitogens (e.g., IL-4, IL-15), suggesting that Noxa induction is linked to proliferation and may serve as a safeguard against oncogenic transformation (Alves et al., 2006).

Expanding on the link between Noxa and glucose metabolism, Lowman et al. found that Noxa is phosphorylated at Ser13 (a residue outside of Noxa’s BH3 domain) by Cdk5 only when cells are in the presence of glucose (Lowman et al., 2010). This phosphorylation causes sequestration of Noxa in the cytosol and suppression of its apoptotic function. Despite not having apoptotic activity, phosphorylated Noxa still associates with the antiapoptotic Bcl-2 family member, Mcl-1 (this observation corroborates earlier observations that the Noxa/Mcl-1 interaction persists in proliferating T cells with high glycolytic activity [Alves et al., 2006]). In addition, glucose stimulates a marked change in the association of Noxa with high-molecular-weight complexes that also harbor Mcl-1 (Lowman et al., 2010). The traditional view of Noxa’s proapoptotic effect is that Noxa, when induced/activated, binds Mcl-1 and promotes its degradation, thus sensitizing cells to MOMP. However, under high-glucose conditions, Noxa binds, but it does not promote Mcl-1 degradation or sensitization to MOMP. Furthermore, Jurkat cells overexpressing Noxa showed inordinately high rates of glucose metabolism, and while prolonged Noxa expression ultimately resulted in Jurkat cell death in stagnant media (in which glucose is depleted over time), the continual supplementation of cells with excess glucose abrogated Noxa-induced death (Lowman et al., 2010). The latter result presumably occurred due to enforcement of continued phosphorylation at Ser13. Together, these observations suggested that the change in Noxa observed by gel filtration might represent its shift from a prodeath to a glycolysis-promoting protein complex, in a scenario very reminiscent of its BH3-only relative Bad. However, while Bad promotes glycolysis when phosphorylated at Ser155, phosphorylated Noxa appears to decrease glycolysis despite increasing glucose consumption. This counterintuitive observation is likely explained by a mechanism wherein Noxa diverts glucose away from glycolysis and into the PPP, as suggested by experiments in which Noxa promoted the conversion of glucose to ribose-5-phosphate (Lowman et al., 2010). This observation is particularly striking, as this mode of metabolism, which generates NADPH and reduced glutathione, promotes suppression of oxidative stress (Ralser et al., 2007)—a far cry from Noxa’s classical role as an apoptosis inducer.
Together, these studies illustrate the emerging dual nature of the BH3-only proteins in regulating both apoptosis and metabolism. For future studies, it will be important to consider whether the interplay between BH3-only proteins and antiapoptotic Bcl-2 proteins (traditionally considered primarily in the context of apoptosis) might have unanticipated metabolic consequences. Indeed, several recent studies have described metabolic functions for Bcl-xL and Mcl-1.

### Antiapoptotic Bcl-2 Proteins

The activation of Bax/Bak and the consequent release of cytochrome c through MOMP are held in check by the antiapoptotic Bcl-2 family proteins Bcl-2, Bcl-xL, Bcl-W, and Mcl-1. The traditional view is that, under stress, BH3-only proteins antagonize the antiapoptotic Bcl-2 proteins, thereby allowing for Bax/Bak activation and MOMP (reviewed in Youle and Strasser, 2008). While this conceptually simple model has been supported by years of research, an expanded view of Bcl-2 protein function has come from recent studies on mitochondrial dynamics. Expression of CED-9, a Bcl-2-like protein from *C. elegans*, can induce mitochondrial fusion (Yamaguchi et al., 2008) and has been shown to interact with core mitochondrial fusion/fission machinery (Lu et al., 2011; Yamaguchi et al., 2008). Furthermore, expression of Mcl-1 and Bcl-xL in mammalian cells can induce mitochondrial fusion (Delivani et al., 2006; Perciavalle et al., 2012; Sheridan et al., 2008), and the *Drosophila* Bcl-2 homolog (Buffy) promotes mitochondrial remodeling during oogenesis (Tanner et al., 2011). These observations raise a question concerning the fundamental mechanism by which Bcl-2 proteins modulate apoptosis: do Bcl-2 proteins modulate apoptosis by controlling mitochondrial fission/fusion? Although mitochondrial fission occurs concomitantly with MOMP, and it seems highly plausible that Bcl-2 stimulation of mitochondrial fission might suppress MOMP, a consensus answer to this question has not emerged (see a recent review on this topic [Martinou and Youle, 2011]). That said, these changes in mitochondrial morphology suggest a metabolic role for antiapoptotic Bcl-2 proteins that may be linked to the mechanism by which they suppress apoptosis.

Several recent studies have implicated antiapoptotic Bcl-2 proteins in the regulation of metabolism. An NMR- and mass spectrometry-based comparison of Bcl-xL-overexpressing and control cells revealed profound differences in metabolite levels (Yi et al., 2011). Notably, Bcl-xL expression reduced the levels of glucose-derived citrate, which consequently led to lower cytosolic acetyl-CoA levels. In turn, low acetyl-CoA levels were linked to a decrease in the N-alpha-acetylation of several proapoptotic proteins, including Bax and caspase-2. For caspase-2, N-alpha-acetylation was shown to be important for its activation (Yi et al., 2011). While the antiapoptotic effect of Bcl-xL is often attributed to its binding and suppression of Bax, mutants of Bcl-xL that are unable to bind Bax still retain up to 80% of their antiapoptotic activity (Cheng et al., 1996). These same Bax-binding mutants still suppress acetyl-CoA levels, suggesting that modulation of metabolism and, in turn, acetyl-CoA levels may account for a significant portion of Bcl-xL’s antiapoptotic activity (Yi et al., 2011).

Bcl-xL has commonly been considered an OMM-associated protein. Numerous studies have characterized its suppression of Bax/Bak at this locale. Additional studies have even implicated Bcl-xL in controlling metabolite and ion permeability of the OMM (Basañez et al., 2002; Lam et al., 1998; Vander Heiden et al., 2001). Two recent studies expand this paradigm by describing a role for Bcl-xL at the IMM in promoting ATP generation (Alavian et al., 2011; Chen et al., 2011). Surprisingly, overexpression of Bcl-xL in neurons increases ATP generation but lowers mitochondrial oxygen consumption (Alavian et al., 2011). While this observation initially seemed paradoxical, an explanation appears to be found in the process of oxidative phosphorylation: H+ protons pumped out of the mitochondrial matrix can re-enter the matrix through ATP synthase (generating ATP) and through a nonproductive H+ leak. Cristae-localized Bcl-xL appears to reduce the nonproductive leakage of H+ into the matrix by directly modulating an ion channel at the IMM, thereby channeling more protons through ATP synthase to generate ATP. Bcl-xL also interacts with the β subunit of F1Fo ATP synthase and increases ATP synthase enzymatic activity both in cells and with purified recombinant enzymes in vitro (Alavian et al., 2011; Chen et al., 2011). Still, however, it is unclear whether the direct modulation of ATP synthase activity accounts for the reduction in H+ leakage.

Most relevant to this review is the question of whether the antiapoptotic effect of Bcl-xL is linked to its role in modulating ATP production/metabolism. As mentioned above, the metabolic and antiapoptotic properties of Bcl-xL cosegregate in Bcl-xL mutants deficient for Bax/Bak binding (Yi et al., 2011). Furthermore, two additional experiments suggest a role for metabolic modulation in Bcl-xL’s antiapoptotic effect: Bcl-xL overexpression protected yeast from heat-induced death but failed to protect yeast deficient in the β subunit of F1Fo ATP synthase (Chen et al., 2011). Moreover, Bcl-xL overexpression protects Bax/Bak double knockout mouse embryonic fibroblasts from death induced by replacing media glucose with galactose, which forces cells to rely on oxidative phosphorylation (rather than glycolysis) for energy (Alavian et al., 2011). In this scenario, Bcl-xL-mediated enhancement of oxidative phosphorylation may help these cells keep pace, at least for a time, with energy demands.

Together, these observations indicate that Bcl-xL can promote survival in a nontraditional, Bax/Bak-independent manner. Indeed, it seems likely that in the face of increased energy demands on the mitochondria (i.e., in galactose-containing media), the Bcl-xL-induced enhancement of ATP production and suppression of a detrimental H+ ion leak would maintain mitochondrial membrane potential. Conversely, in the absence of Bcl-xL, the nonproductive leakage of H+ ions and weakened ATP synthase activity would likely prevent mitochondria from keeping pace with energy demand, potentially resulting in mitochondrial depolarization regardless of Bax/Bak status (Chen et al., 2011).

How might this view of Bcl-xL function jibe with the long-held view that Bcl-xL directly suppresses apoptosis by inhibiting Bax/Bak-mediated cytochrome c release? The relative contribution of either potential death-suppressing mechanism—Bcl-xL suppression of Bax/Bak versus modulation of metabolism—may depend on where Bcl-xL is predominately localized.
(OMM versus cristae) in a given cell type. Alternatively, these two functions of Bcl-xL may be related: ATP synthase activity has been linked to Bax activation (Matsuyama et al., 1998). In any case, the observation that mutants of Bcl-xL deficient for Bax/Bak binding still suppress apoptosis (albeit to a slightly lesser degree than WT) yet maintain their effect on metabolism clearly argues for an expansion of the Bcl-xL paradigm to include modulation of metabolism as part of its antiapoptotic function.

In an intriguing parallel to Bcl-xL, Mcl-1 has recently been shown to reside in the mitochondrial matrix (Huang and Yang-Yen, 2010; Perciavalle et al., 2012) and have a role in promoting both ATP production and maintenance of mitochondrial membrane potential (Perciavalle et al., 2012). Perciavalle and colleagues found that inducible deletion of Mcl-1 results in gross defects in both IMM architecture and mitochondrial fusion. A low-molecular-weight isoform of Mcl-1, generated by an N-terminal cleavage, was found to reside in the mitochondrial matrix; its expression restored normal IMM morphology and mitochondrial dynamics. Furthermore, this Mcl-1 isoform is necessary for normal mitochondrial respiration and ATP production, and its expression enhances the assembly of F1F0-ATP synthase oligomers (Perciavalle et al., 2012). The latter observation, in particular, is similar to the emerging picture of Bcl-xL. However, while the antiapoptotic function of Bcl-xL, appears to be tied to its role in promoting mitochondrial metabolism, the matrix-localized metabolism-modulating species of Mcl-1 fails to protect cells from chemotherapy-induced apoptosis (Perciavalle et al., 2012). In contrast, a mutant of Mcl-1 that localizes to the OMM, but not the matrix, maintains its antiapoptotic function (Perciavalle et al., 2012). Thus, Mcl-1, while performing metabolic functions inside the mitochondria, seems to conform to the canonical view of antiapoptotic Bcl-2 proteins by inhibiting apoptosis at the level of Bax/Bak on the mitochondrial surface.

Prior to this recent work on Mcl-1’s function in the matrix, Mcl-1 had been characterized as a metabolically controlled protein: Mcl-1 protein stability is affected by the presence of glucose, such that in the absence of glucose, glycogen synthase kinase-3 (GSK3) phosphorlates and targets Mcl-1 for degradation via the proteasome. Conversely, the stimulation of glucose uptake by growth factors leads to the inhibition of GSK3 and consequent stabilization of Mcl-1 (Maurer et al., 2006; Zhao et al., 2007). While in the context of apoptosis the stabilization of Mcl-1 had been considered to be a mechanism to protect growth factor-stimulated cells, the metabolic consequences of glucose-mediated Mcl-1 stabilization have yet to be explored. Given that growth factor stimulation induces cell proliferation, which carries with it the need for increased biosynthetic metabolism and efficient mitochondrial energy production, it seems plausible that glucose-mediated Mcl-1 stabilization might serve the metabolic needs of proliferating cells. In addition, determining whether the ratio of OMM- to matrix-localized Mcl-1 depends on circumstances (e.g., proliferation versus quiescence) may be illuminating, as this ratio may be dictated by the metabolic demands of a given cell.

**Metabolic Regulation of Apoptosis Upstream of the Bcl-2 Family**

**The Tumor Suppressor p53**

As one of the most-studied proteins in the cell, the tumor suppressor p53 has been extensively characterized as a stress-induced, proapoptotic protein. Its most well-characterized role in apoptosis is as a transcriptional activator of certain proapoptotic Bcl-2 family proteins, including Bax, Puma, and Noxa. In addition, p53 can act in the cytosol as a direct activator of MOMP by engaging directly with Bax/Bak (Chipuk et al., 2004). Adding new levels of complexity to p53, recent work suggests that p53's role in cell stress is quite nuanced, such that the p53-dependent response to stress can vary from apoptosis, to senescence, autophagy, or metabolic adaption (reviewed in detail by Gottlieb and Vousden, 2010).

p53 is activated by metabolic stress. Under low-nutrient conditions, the activation of AMP-activated protein kinase (AMPK) induces both p53 transcription and stabilization of p53 protein (Jones et al., 2005; Okoshi et al., 2008). p53 induction in this scenario appears to result in the general activation of catabolic pathways in order to sustain energy levels under low-nutrient conditions. More specifically, accumulating evidence suggests that p53 can impact several metabolic pathways to tailor a cell’s response to this metabolic stress (Gottlieb and Vousden, 2010): p53 inhibits glycolytic flux by suppressing the expression of the insulin receptor and GLUTs (Kawauchi et al., 2008; Schwarzenberg-Bar-Yoseph et al., 2004). Additionally, activation of p53 results in the induction of TP53-induced glycolysis and apoptosis regulator (TIGAR), which functions as a fructose-2,6-bisphosphatase. Thus, TIGAR antagonizes the third step in glycolysis, the conversion of fructose-6-phosphate to fructose-2,6-bisphosphate (see Figure 1). Blockage at this step causes an accumulation of fructose-6-phosphate, which can be isomerized back to G6P to promote PPP activity. Indeed, the net metabolic effect of TIGAR induction seems to be the rerouting of glucose into the oxidative arm of the PPP, which generates reduced glutathione and neutralizes toxic ROS. Consistent with this, TIGAR expression leads to suppression of ROS-induced cell death (Bensaad et al., 2006). In addition to its effects on glycolysis, p53 promotes oxidative phosphorylation (Matoba et al., 2006; Okamura et al., 1999; Stambolsky et al., 2006) and thereby seems to favor energy production through the TCA cycle, as opposed to glycolysis. Furthermore, p53 promotes the use of glutamine as a metabolic fuel (Hu et al., 2010; Suzuki et al., 2010) and promotes the oxidation of fatty acids (Buzzi et al., 2007; Ide et al., 2009), both potential adaptive responses to promote TCA cycle-mediated generation of energy during periods of nutrient scarcity.

These observations clearly expand the circle of p53 influence beyond traditional associations with DNA damage and apoptosis induction, and implicate it as a pivotal switch in cellular metabolism. Notably, the p53-induced pattern of metabolic regulation (suppression of glycolytic flux, upregulation of TCA cycle) is in stark contrast to the high levels of aerobic glycolysis (Warburg effect) commonly observed in cancerous tissue, suggesting that the deletion of p53 in tumors may serve a metabolic function (Gottlieb and Vousden, 2010). Additionally, the
p53-regulated metabolic adaptations described above appear to cast p53, at least under certain circumstances, as a survival factor. In this regard, it is interesting to note that p53 has been shown to both promote and antagonize the induction of autophagy (Crighton et al., 2006; Scherz-Shouval et al., 2010; Tassdemir et al., 2008; Yee et al., 2009), a prosurvival process by which cells recycle their own organelles and protein to be used as fuel during periods of nutrient scarcity, again suggesting that p53 may toggle between promoting survival or cell death, likely depending on the intensity and/or duration of the stress (Maddocks and Vousden, 2011; Scherz-Shouval et al., 2010).

**Sirtuins, Metabolism, and Cell Death**

Sirtuins are a family of lysine deacetylases and mono-ADP ribosyltransferases (Sirt1-7 in mammals) that use NAD+ as a cosubstrate to target numerous cellular proteins (for review, see Guarente, 2011). As a group, Sirtuins have been implicated in several diseases, including cancer, metabolic syndrome, and Alzheimer’s disease, and are thought to mediate many of the metabolic effects of caloric restriction (Guarente, 2011). Given their dependence on NAD+, a metabolite and hydride-transferring cofactor in the redox reactions of metabolism, Sirtuins are an intriguing signaling link between cell fate and metabolism.

Five of the seven Sirtuins (Sirt1, Sirt3, Sirt4, Sirt5, and Sirt6) have been reported to modulate metabolic pathways (reviewed in Li and Kazarin, 2011). Sirt1, in particular, seems to have wide-ranging metabolic effects. In response to long-term caloric restriction, Sirt1 increases hepatic glucose levels (via gluconeogenesis), stimulates fatty acid oxidation, and inhibits lipogenesis, while the mitochondria-localized Sirt3 enhances mitochondrial lipid catabolism and stimulates the urea cycle (reviewed in Chalkiadaki and Guarente, 2012). Additionally, Sirt1 has been heavily implicated in the regulation of cell death. Sirt1 suppresses cell death through the modulation of forkhead-dependent transcriptional pathways in response to oxidative stress and nutrient deprivation (Brunet et al., 2004; Motta et al., 2004), and a similar function was reported for Sirt2 (Wang et al., 2007). Sirt1 also promotes survival by deacetylating and suppressing the nuclear translocation of p53, which, in turn, prevents p53 from upregulating its proapoptotic target genes (Luo et al., 2001; Vaziri et al., 2001).

Like Sirt1, Sirt3 has been characterized as a modulator of metabolism. Among its metabolic functions, Sirt3 promotes oxidative phosphorylation by deacetylating complexes I and II (Ahn et al., 2008; Cimen et al., 2010) and promotes fatty acid oxidation, under fasting conditions, by deacetylating long chain-specific acyl-CoA dehydrogenase (LCAD) (Hirshey et al., 2010). Additionally, Sirt3 deacetylates and promotes the activity of Mn superoxide dismutase (MnSOD), a mitochondrial ROS scavenger (Tao et al., 2010). Interestingly, stimulation of MnSOD may counterbalance the ROS produced as a result of the Sirt3-mediated increase in oxidative phosphorylation. In line with a role in suppressing oxidative stress, Sirt3 deacetylates and activates mitochondrial isoform dehydrogenase (IDH2), leading to the production of NADPH. This function of Sirt3 protects cells from oxidative damage in a mouse model of age-related hearing loss (Someya et al., 2010).

Taken alone, the antioxidant functions of Sirt3 cast it as a prosurvival enzyme, a characteristic associated with oncogenes. However, higher levels of ROS caused by Sirt3 deletion appear to be linked to genomic instability, which renders cells more susceptible to oncogenic transformation (Kim et al., 2010; Tao et al., 2010). In addition, Sirt3 expression is commonly lost in tumors (Finley et al., 2011), and deletion of Sirt3 appears to promote aspects of cancer metabolism, as Sirt3−/− MEFs display increased glycolysis and decreased oxidative phosphorylation (Finley et al., 2011; Kim et al., 2010). Furthermore, depletion of Sirt3 in tissue culture cells is reported to increase the association of hexokinase II to mitochondria and thereby block the activation of Bax-mediated MOMP (Verma et al., 2012). Together, the metabolic and apoptosis-regulating functions of Sirt3 have begun to frame this enzyme as a tumor suppressor.

While Sirtuin activity can be modulated by phosphorylation (North and Verdin, 2007; Sasaki et al., 2008), NAD+ levels seem to play a central role, such that turning NAD+ levels up or down is sufficient to trigger a corresponding rise or fall in Sirtuin activity (Imai, 2009; Revollo et al., 2004). Therefore, as varying nutrient levels cause fluctuations in NAD+, Sirtuins are poised to translate these changes to the molecular level by modifying the function of their substrates (Guarente, 2011), resulting in the modulation of cell survival and metabolism pathways described above. Thus, the connection between NAD+ metabolism and the cell fate-modulating functions of Sirtuins represents a clear example of metabolism-to-cell-fate crosstalk.

In mammals, NAD+ can be generated de novo from L-tryptophan and/or through salvage pathways, one of which involves the rate-limiting nicotinamide phosphoribosyltransferase (NAMPT), an enzyme that has gained notoriety as a potential anticancer target (Bi and Che, 2010). The NAMPT-mediated salvage pathway is the primary source of NAD+ synthesis in mammals (Collins and Chaykin, 1972; Rongvaux et al., 2003), and NAMPT expression is increased by caloric restriction, resulting in an increase in mitochondrial NAD+ levels (Yang et al., 2007). Importantly, the NAMPT-mediated increase in mitochondrial NAD+ causes marked resistance to cell death, which requires the mitochondria-localized Sirtuins, Sirt3 and Sirt4 (Yang et al., 2007). The posttranslational modification (PTM) that most Sirtuins antagonize, lysine acetylation, is also metabolite-derived, from acetyl-CoA, suggesting that different modes of metabolism may communicate to cell death effectors both via the regulation of enzymes (e.g., NAD+) and more directly through regulating the availability of donor substrates (e.g., acetyl-CoA). Indeed, simply increasing the levels of cellular acetyl-CoA (by the addition of citrate to cells, for example) is sufficient to modulate protein acetylation and affect cell survival (Wellen et al., 2008; Yi et al., 2011). In addition to acetylation, recent studies show that Sirt5 antagonizes lysine succinylation and malonylation (Du et al., 2011; Peng et al., 2011), which, like acetylation, are metabolite (acyl-CoA)-derived modifications. Furthermore, other metabolite-derived PTMs, such as glycosylation and lipidation, likely also influence core cellular pathways. Indeed, in response to tumor cell hypoxia, glycosylation was recently shown to suppress phosphofructokinase-1 (PFK1), leading to a redirection of glucose through the PPP and the consequent generation of...
NADPH. In this context, an increase in PPP-generated NADPH protected cells from oxidative stress-induced cell death (Yi et al., 2012). Although the precise functions and breadth of many of these PTMs are still emerging, it underscores how fluctuations in metabolite levels (e.g., acyl-CoA, glucose, N-acetyl-D-glucosamine) may directly communicate to cell fate pathways (for more detail, we recommend a recent review on this topic [Wellen and Thompson, 2012]).

Caspase-2

Despite being one of the most evolutionarily conserved caspases, caspase-2 continues to be somewhat enigmatic. Its precise function and the contexts in which it plays a central role in promoting apoptosis have been hard to pin down. These issues have been well documented in various reviews on the topic (Bouchier-Hayes, 2010; Krumschnabel et al., 2009a, 2009b), and recent work suggests that caspase-2 may have novel functions in promoting antioxidant defense (Shalini et al., 2012) and regulating cell-cycle checkpoints (Ho et al., 2009; Kumar, 2009; Oliver et al., 2011). In the apoptotic cascade, caspase-2 functions as an apical caspase, upstream of MOMP, that cleaves and activates Bid (generating tBid) to promote Bax/Bak activation (Nutt et al., 2005; Bonzon et al., 2006; Bouchier-Hayes et al., 2009; Harvey et al., 1997).

Strikingly, however, the only overt developmental defect observed in the caspase-2 knockout mouse was an overabundance of oocytes, suggesting that caspase-2 may play a prominent role in this tissue (Bergeron et al., 1998; Morita and Tilly, 1999).

Building on the oocyte-caspase-2 connection, studies in the Xenopus egg/oocyte system have shown that caspase-2 activation is metabolically controlled. Depletion of metabolites in egg extracts over time leads to spontaneous “cell-free” apoptosis, characterized by caspase-2 activation, MOMP, and downstream caspase activation. Supplementation of egg extracts with metabolites (e.g., G6P) suppresses caspase-2 activation by triggering a CamKII-dependent phosphorylation within the caspase-2 prodomain (Ser135, Xenopus numbering) (Nutt et al., 2005). The Ser135 phosphorylation is protected from dephosphorylation by promoting the interaction between 14-3-3z and caspase-2 (Figure 3).

Figure 3. Model for the Metabolic Regulation of Caspase-2 in Xenopus Eggs/Oocytes

High NAD+ levels stimulate Sirt1 deacetylase activity, which, in turn, activates prosurvival transcriptional pathways. Additionally, Sirtuin deacetylase activity maintains 14-3-3z in a deacetylated state, permitting binding between 14-3-3z and caspase-2. As NAD+ levels drop, Sirtuin activity diminishes and 14-3-3z becomes acetylated at lysine residues critical for protein-protein interactions. This disrupts 14-3-3z/caspase-2 binding, and frees caspase-2 to become active in response to a stress stimulus.

Conclusion

The decision of a cell to live or die by apoptosis is clearly influenced by the availability of nutrients and the metabolic pathways active within the cell. In light of this, it may not be completely unexpected that Bcl-2 proteins and other regulators of apoptosis have dual metabolic and apoptotic functions and in some cases, such as Bcl-xL and Noxa, may exert their effect on cell death, at least in part, by regulating metabolism. Furthermore, this emerging theme of Bcl-2 protein duality may only represent a small fraction of crosstalk between metabolic and apoptotic pathways, as metabolism feeds back to regulate cell fate-controlling proteins through metabolite-derived PTMs such as acetylation and glycosylation (areas that are still largely unexplored). Indeed, an understanding of how these metabolite-derived PTMs differ across the proteome between diseased and normal tissue and affect apoptotic-signaling pathways will be illuminating. Moreover, as this field is pushed forward, it will be critical to apply our understanding of the metabolism-apoptosis connection to the development of therapeutics for diseases in which metabolic alterations precede the apoptotic loss of critical tissues/cells, as seen in Alzheimer’s disease and diabetes. In this regard, the studies presented here suggest that therapeutic intervention to prevent metabolic alterations in these tissues may be an effective way to prevent downstream tissue degeneration via apoptosis (rather than therapeutically...
targeting apoptosis itself). Conversely, the development of metabolism-targeted therapies to enhance cell death in tumors is well under way. All together with the innumerable metabolites and complex circuitry of metabolism, the recent examples of molecular crosstalk between apoptosis and metabolism highlighted here illustrate what will be a very challenging and important field of study for years to come.

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