Metabolic Stress in Autophagy and Cell Death Pathways

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Growth factors and oncogenic kinases play important roles in stimulating cell growth during development and transformation. These processes have significant energetic and synthetic requirements and it is apparent that a central function of growth signals is to promote glucose metabolism to support these demands. Because metabolic pathways represent a fundamental aspect of cell proliferation and survival, there is considerable interest in targeting metabolism as a means to eliminate cancer. A challenge, however, is that molecular links between metabolic stress and cell death are poorly understood. Here we review current literature on how cells cope with metabolic stress and how autophagy, apoptosis, and necrosis are tightly linked to cell metabolism. Ultimately, understanding of the interplay between nutrients, autophagy, and cell death will be a key component in development of new treatment strategies to exploit the altered metabolism of cancer cells.

Although single-celled organisms grow and proliferate based on nutrient availability, metazoan cells rely on growth factor input to promote nutrient uptake, regulate growth and proliferation, and survive (Raff 1992; Rathmell et al. 2000). Access and competition for these signals are critical in developmental patterning and to maintain homeostasis of mature tissues. Cells that do not receive proper growth factor signals typically atrophy, lose the ability to uptake and use extracellular nutrients, and instead induce the self-digestive process of autophagy as an intracellular energy source before ultimately undergoing programmed cell death. Cancer cells, in contrast, often become independent of extracellular growth signals by gaining mutations or expressing oncogenic kinases to drive

intrinsic growth signals that mimic growth factor input, which can be the source of oncogene addiction. Growth factor input or oncogenic signals often drive highly elevated glucose uptake and metabolism (Rathmell et al. 2000; DeBerardinis et al. 2008; Michalek and Rathmell 2010). First described in cancer by Warburg in the 1920s, this highly glycolytic metabolic program is termed aerobic glycolysis and is a general feature of many nontransformed proliferative cells (Warburg 1956; DeBerardinis et al. 2008).

Nutrient uptake and aerobic glycolysis induced by growth signals play key roles in cell survival (Vander Heiden et al. 2001). Manipulating cell metabolism as a means to promote the death of inappropriately dividing cells, therefore, is a promising new avenue to treat disease.

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Targeting the altered metabolism of cancer cells in particular is of great interest. It is still unclear at the molecular level, however, how inhibiting or modulating cell metabolism leads to apoptosis, and how these pathways may best be exploited (Dang et al. 2009; Wise and Thompson 2010).

Growth factor or oncogenic kinases promote multiple metabolic pathways that are essential to prevent metabolic stress and may be targets in efforts to link metabolism and cell death (Vander Heiden et al. 2001). Decreased glucose metabolism on loss of growth signals leads to decreased ATP generation as well as loss in generation of many biosynthetic precursor molecules, including nucleic acids, fatty acids, and acetyl-CoA for acetylation (Zhao et al. 2007; Wellen et al. 2009; Coloff et al. 2011). Glucose is also important as a precursor for the hexosamine pathway, to allow proper glycosylation and protein folding in the endoplasmic reticulum (Dennis et al. 2009; Kaufman et al. 2010). If glucose metabolism remains insufficient or disrupted, the cells can switch to rely on mitochondrial oxidation of fatty acids and amino acids, which are energy rich but do not readily support cell growth and can lead to potentially dangerous levels of reactive oxygen species (Wellen and Thompson 2010). Amino acid deficiency can directly inhibit components of the signaling pathways downstream from growth factors and activate autophagy (Lynch 2001; Beugnet et al. 2003; Byfield et al. 2005; Nobukuni et al. 2005). Finally, hypoxia induces a specific pathway to increase nutrient uptake and metabolism via the hypoxia-inducible factor (HIF1/2 α) that promotes adaptation to anaerobic conditions, but may lead to apoptosis if hypoxia is severe (Saikumar et al. 1998; Suzuki et al. 2001; Fulda and Debatin 2007).

Typically a combination of metabolic stresses rather than loss of a single nutrient input occur at a given time (Degenhardt et al. 2006) and autophagy is activated to mitigate damage and provide nutrients for short-term survival (Bernales et al. 2006; Tracy et al. 2007; Altman et al. 2011; Guo et al. 2011). Autophagy is a cellular process of bulk cytoplasmic and organelle degradation common to nearly all eukaryotes.

Unique double-membraned vesicles known as autophagosomes engulf cellular material and fuse with lysosomes to promote degradation of the contents (Kelekar 2005). Described in greater detail below, autophagy can reduce sources of stress, such as protein aggregates and damaged or dysfunctional intracellular organelles, and provide nutrients during times of transient and acute nutrient withdrawal.

Despite the protective effects of autophagy, cells deprived of growth signals, nutrients, or oxygen for prolonged times will eventually succumb to cell death. Apoptosis is the initial death response on metabolic stress and is regulated by Bcl-2 family proteins. In healthy cells, antiapoptotic Bcl-2 family proteins, such as Bcl-2, Bcl-xl, and Mcl-1, bind and inhibit the multidomain proapoptotic proteins Bax and Bak (van Delft and Huang 2006; Walensky 2006; Chipuk et al. 2010). In metabolic stress, proapoptotic “BH3-only” proteins of the Bcl-2 family are induced or activated and bind to and inhibit the antiapoptotic Bcl-2 family proteins to allow activation of the proapoptotic Bax and Bak (Galonek and Hardwick 2006). The BH3-only proteins Bim, Bid, and Puma can also directly bind and activate Bax and Bak (Letai et al. 2002; Ren et al. 2010). Active Bax and Bak disrupt the outer mitochondrial membrane (termed mitochondrial outer-membrane permeabilization, or MOMP) and release several proapoptotic factors including cytochrome-C that activate the apoptosome that in turn activates effector caspases to cleave a variety of cellular proteins and drive apoptosis (Schafer and Kornbluth 2006). In cases in which these apoptotic pathways are suppressed, metabolic stress can instead lead to necrotic cell death (Jin et al. 2007).

ROLE OF GROWTH FACTORS IN METABOLISM AND CELL DEATH

The phosphatidylinositol-3-kinase (PI3K)/AKT/mTOR pathway, outlined in Figure 1, is central in growth factor-stimulated control of glucose uptake and metabolism and is often altered in cancer. PI3K catalyzes the phosphorylation of phosphatidylinositol (PI) 4,5 bisphosphate (PIP2) to PI 3,4,5 triphosphate (PIP3). After

Figure 1. The PI3K-Akt-mTORC1 pathway and control of autophagy. PI3K is induced downstream from growth factor input and activates Akt and mTORC1. Akt supports the localization of nutrient transporters to the cell surface and maintains nutrient uptake. mTORC1 both supports protein translation and inhibits autophagy through phosphorylation of ULK1 and ULK2. AMPK, activated under energetic stress, inhibits mTORC1 and directly associates with ULK1/2 to activate autophagy.

growth factor signals are received, PIP3 accumulates, leading to recruitment and phosphorylation of Akt (Frech et al. 1997; Zoncu et al. 2011). Activated Akt supports metabolism in a number of ways, notably by maintaining protein translation, glucose metabolism, and inhibiting autophagy and apoptosis. Akt is essential for growth factor-stimulated glucose uptake and glycolysis by localizing Glut1 to the cell surface (Plas et al. 2001; Vander Heiden et al. 2001; Wieman et al. 2007; Wofford et al. 2008) and also increasing the activity and proper localization of hexokinases (HKs) (Gottlob et al. 2001; Robey and Hay 2006) to support the phosphorylation and downstream catabolism of glucose. Akt can also promote glucose metabolism through the pentose phosphate pathway (PPP), which generates NADPH and ribonucleotides to control cellular redox as well as lipid and nucleic acid synthesis (Rathmell et al. 2003; Duvel et al. 2010). In addition, Akt sup-

presses the catabolism of intracellular components such as fatty acids that would otherwise be used to support cell growth (Deberardinis et al. 2006). Thus, Akt activation promotes glucose metabolism and inhibits other metabolic pathways, leading to a glycolytic phenotype in growth factor-stimulated cells and the aerobic glycolysis characteristic of cancer cells (Elstrom et al. 2004). Importantly, Akt is highly anti-apoptotic, but requires glucose to protect cells from death (Vander Heiden et al. 2001; Rathmell et al. 2003; Coloff et al. 2011), highlighting the connections between metabolism and apoptosis and enforcing a glucose addiction on stimulated or cancerous cells.

mTORC1 (mechanistic target of rapamycin complex 1) is activated downstream from Akt and is directly regulated by amino acid availability (Lynch 2001; Beugnet et al. 2003; Rocco et al. 2006; Kim et al. 2008; Sancak et al. 2010) to control translation and autophagy (Zoncu

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et al. 2011). mTORC1 regulates cell growth by promoting protein translation through phosphorylation of two key enzymes. Protein synthesis inhibitor eIF4E binding protein (4EBP) is inactivated by mTORC1 to allow for cap-dependent translation, and S6 kinase (S6K) is phosphorylated and activated by mTORC1 to activate the S6-ribosomal protein that appears to be important for cell metabolism and growth (Hara et al. 1998; Fingar et al. 2002; Tandon et al. 2011). mTORC1 also negatively regulates autophagy through inhibitory phosphorylation of the autophagy-essential kinases Unc-51-like kinase-1 (ULK1) and ULK2 (Ganley et al. 2009; Jung et al. 2009). In addition, mTOR signaling can be suppressed and autophagy induced by the AMP-activated protein kinase (AMPK) (Hoyer-Hansen and Jaattela 2007a; Canto and Auwerx 2010).

Many oncogenes can mimic growth signals to support metabolism in the absence of exogenous growth factor signaling (Vander Heiden et al. 2009). The PI3K/Akt/mTOR pathway plays a major role downstream from these oncogenic mutations to drive aerobic glycolysis. The BCR-Abl fusion protein, for example, promotes trafficking of Glut1 to the cell surface. Inhibition of PI3K, however, blocks this function and Glut1 is internalized thus limiting glucose uptake and glycolysis (Barnes et al. 2005). Myc can directly promote transcription of essentially all key glycolytic genes (Osthus et al. 2000; Dang et al. 2009). Indeed, recent studies have shown that c-Myc and Akt have complementary effects in promoting aerobic glycolysis (Fan et al. 2010). In addition to glucose, proliferating cells also require glutamine (Wise et al. 2008; Dang et al. 2009) and c-Myc has been shown to drive glutamine uptake and metabolism to render cells dependent on this important nutrient to support energy generation and biosynthesis (Wise et al. 2008; Gao et al. 2009).

AUTOPHAGY AND CONTROL OF METABOLIC STRESS

Decreased metabolism leads to induction of autophagy to generate nutrients from intracellular components until proper extracellular nutrient

uptake is restored. Autophagy can play a pro-death role when prolonged or in certain developmental conditions, such as elimination of blastocyst inner cell mass (see review by Das et al. 2012), but in most circumstances autophagic generation of nutrients prevents or delays cell death. In autophagy, the cell packages organelles, bulk cytoplasm, and long-lived proteins in double-membraned vesicles for delivery to the lysosome and eventual degradation (Kelekar 2005). Autophagy occurs in all metazoan cells at low levels under basal conditions as a quality-control and waste-disposal mechanism (Ganley et al. 2009; Jung et al. 2009), but is induced as a protective mechanism when cells are under stress (Fig. 2).

Induction of autophagy can relieve a variety of cell stresses (Fig. 2). Autophagy can prevent overaccumulation of mitochondria and remove damaged mitochondria to prevent cell death (Colell et al. 2007; Pua et al. 2009) and to prevent an increase in damaging reactive oxygen species (ROS) (Mathew et al. 2009; Rouschop et al. 2009; Tal et al. 2009). Although some amounts of ROS are required for normal cell signaling (Hamanaka and Chandel 2010), excess ROS can lead to cellular damage, p53 activation, induction of proapoptotic proteins, and cell death in a wide variety of tissues (von Harsdorf et al. 1999; Sade and Sarin 2004; Karawajew et al. 2005; Liu et al. 2008a; Niizuma et al. 2009; Bodet et al. 2010). Autophagy is also important to engulf damaged endoplasmic reticulum (ER) in the unfolded protein response (Bernales et al. 2006; Hoyer-Hansen and Jaattela 2007b) and degrade protein aggregates that may otherwise lead to neurodegeneration (Bjorkoy et al. 2005; Komatsu et al. 2006). Finally, autophagy is essential to limit DNA damage and genomic instability, possibly through modulation of protein aggregates and the adaptor protein p62 (Mathew et al. 2007, 2009). As a consequence, autophagy-deficient cancer cells have increased genomic damage that can paradoxically promote tumor progression (Liang et al. 1999; Yue et al. 2003). This DNA damage may lead to a p53-dependent stress response, and p53 deficiency can promote continued proliferation despite insufficient autophagy and DNA damage (Altman et al. 2011).

Figure 2. Autophagy responds to a variety of stresses to prevent cell death. Autophagy can be induced by a variety of nutrient stresses and other cellular insults. Induced autophagy can provide nutrients for mitochondrial oxidation, suppress p53 pathway activation, and ultimately delay apoptosis.

As discussed above, growth factor-mediated activation of the PI3K-Akt-mTORC1 pathway suppresses autophagy in healthy cells in nutrient-replete conditions. mTORC1 inhibits autophagy by phosphorylating and inhibiting ULK1 and ULK2, key upstream regulators of autophagy (Fig. 1) (Ganley et al. 2009; Jung et al. 2009). Growth factor or nutrient withdrawal (particularly withdrawal of amino acids) inactivates mTORC1 and activates autophagy (Beugnet et al. 2003; Altman et al. 2009). The program of autophagy is performed by a complex pathway of Atg-family proteins and two ubiquitinlike conjugation systems, reviewed in detail in Das et al. (2012). In addition to amino acid-sensitive regulation of autophagy by mTORC1, decreases in ATP resulting from nutrient deprivation can activate the AMPK pathway to promote autophagy. The AMPK heterotrimer is maximally activated when both AMP (adenyl monophosphate) increases and the AMPK α subunit is phosphorylated by the tumor suppressor LKB1 (Hawley et al. 2003; Woods et al. 2003; Shaw et al. 2004; Shaw 2009). AMPK then initiates a cellular program to conserve and generate additional ATP by initiating a G1 cell-cycle arrest, increasing glucose uptake, glycolysis, fatty acid oxidation, and halting protein synthesis and glycogen synthesis (Imamura et al. 2001; Jones et al. 2005; Shaw 2009). AMPK

can phosphorylate and activate TSC2 to inhibit mTORC1 and thus indirectly activate autophagy to degrade damaged organelles and mobilize intracellular nutrients (Hoyer-Hansen and Jaattela 2007a; Canto and Auwerx 2010). In addition, several recent studies in *Caenorhabditis elegans* and mammalian cells have shown that AMPK directly associates and phosphorylates ULK1 in response to glucose deprivation or multinutrient deprivation, and that this interaction is essential for induction of autophagy (Fig. 1) (Egan et al. 2011; Kim et al. 2011; Shang et al. 2011).

Autophagic degradation of mitochondria and other organelles, protein aggregates, cytoplasm, and long-lived proteins can yield significant energy to cells (Kelekar 2005). Autophagosomes fuse with lysosomes to digest the contents and release amino acids and free fatty acids back to the cytosol for mitochondrial oxidation (Fig. 2) (Altman et al. 2011; Guo et al. 2011). This metabolic strategy can be highly efficient to maintain bioenergetics for a potential long period, as complete catabolism of a single molecule of palmitate, a long-chain fatty acid that is often a component of the phospholipid bilayer of membranes, can yield up to 104 ATP, compared to 31 from a single molecule of glucose (Salway 2004). Likewise, metabolism of amino acids can also yield large amounts of ATP.

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Although a role for autophagy as a nutrient source has been apparent in a variety of genetic experiments using yeast (Abeliovich and Klionsky 2001), direct biochemical evidence for autophagy in mammalian cell metabolism has emerged only recently. Lum et al. (2005) showed that autophagy eventually becomes critical as a nutrient source for survival after growth factor deprivation of Bax/Bak-deficient cells. Most interestingly, this cell death caused by autophagy inhibition could be rescued by addition of exogenous nutrients, strongly indicating that autophagy was functioning as a nutrient-generating process. Similarly, Boya et al. (2005) showed that autophagy was critical for survival under conditions of nutrient limitation. More recently, we examined the potential of autophagy to support metabolism by mass spectrometry measurement of metabolite levels in autophagy-deficient cells. These data highlighted the ability of autophagy to supply growth factor or nutrient-deprived cells with an intracellular source of long-chain fatty acids (Altman et al. 2009, 2011). Likewise, Singh et al. (2009) showed that autophagy is critical in regulating lipid metabolism. This ability of autophagy to produce nutrients can be critical to allow cell survival in conditions of low exogenous nutrients (Degenhardt et al. 2006; Karantza-Wadsworth et al. 2007; Mathew et al. 2007; Altman et al. 2011).

The ability of autophagy to both control cell stress and produce nutrients has led to the recent observation that some cancers may be “addicted” to autophagy. We found that autophagy may suppress cell stress and a p53-dependent pathway of cell death, and that cells transformed by the BCR-Abl oncogene were dependent on autophagy for survival and leukemogenesis (Altman et al. 2011). Similarly, Guo et al. (2011) recently showed that autophagy is necessary for survival and can support mitochondrial metabolism of Ras-transformed cells under conditions of nutrient starvation. In Ras-transformed breast epithelial cells, Lock et al. showed that autophagy increases glucose flux through glycolysis to support survival of matrix-detached cells (Lock et al. 2011). These data all suggest that autophagy can prevent death in the absence of nutrients in part by

supporting metabolism from breakdown of intracellular components and may play a broad role in cellular homeostasis and control of stress.

Despite these effects to relieve stress, autophagy induction can in some cases lead to apoptosis rather than protection from cell death. In *Drosophila*, Scott et al. (2007) showed that enforced expression of Atg1, the homolog of ULK1/2, led to apoptosis. Autophagy contributes to cell death of several different tissue types during *Drosophila* development, including the salivary gland, midgut, and reproductive cells (Berry and Baehrecke 2007; Hou et al. 2008; Denton et al. 2009; Nezis et al. 2010). We observed in several mammalian hematopoietic lines that autophagy induction in the absence of growth factor initially provided nutrients but eventually led to apoptosis dependent on direct induction of the proapoptotic protein Bim (Altman et al. 2009). Similarly, Kiyono et al. (2009) recently observed that autophagy induced in hepatocellular carcinoma cells by TGF β led to induction of Bim and the proapoptotic protein BMF and eventual apoptosis. Finally, other groups showed that autophagy was necessary for apoptosis of rat neurons treated with a glutamate receptor agonist (Wang et al. 2008a) and for apoptosis downstream from death receptor signaling mouse embryonic fibroblasts (Wang et al. 2008b). However, the molecular mechanisms governing the decision of autophagy to act as a cytoprotective process or to induce apoptosis are poorly understood.

BEYOND AUTOPHAGY: METABOLIC STRESS AND APOPTOSIS

When autophagy is unable to provide sufficient additional nutrients or mitigate cell stress, insufficient metabolism can induce apoptosis. Links between metabolism and apoptosis are now widely appreciated in many species, and glucose metabolism has been shown to play a direct role in regulation apoptosis. In *Xenopus* oocytes, nutrient depletion over time leads to reduced NADPH and activation of caspase 2 to induce apoptosis (Nutt et al. 2005, 2009). Likewise, loss of growth signals and diminished Akt/

hanced cell survival (Coloff et al. 2011). Bim expression is also increased when glucose metabolism is blocked, although this may be owing to the onset of ER stress (Puthalakath et al. 2007) and deficiency of the hexosamine pathway rather than an energetic stress.

In contrast to Puma and Bim, the proapoptotic Bcl-2 family protein Noxa is important to promote apoptosis in metabolic stress, but Noxa activity rather than expression appears regulated by cell metabolism. Eldering and colleagues showed in two separate studies that activated T cells up-regulated Noxa, which bound to the antiapoptotic protein Mcl-1. When glucose became limiting, Mcl-1 levels decreased and Noxa promoted apoptosis (Alves et al. 2006; Wensveen et al. 2011). Lowman et al. shed some light on a possible mechanism for this effect by demonstrating that glucose deprivation led to loss of an inhibitory phosphorylation of Noxa, promoting Noxa activation (Lowman et al. 2010). Ultimately the combination of proapoptotic proteins including Puma, Noxa, and also Bim all contribute to cell death.

AMP and LKB1 can activate AMPK on metabolic stress to regulate Bcl-2 family proteins and apoptosis. AMPK and LKB1 can inhibit apoptosis by promoting up-regulation of Bcl-

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xL and suppressing proapoptotic Erk signaling (Cao et al. 2010; Kim et al. 2010). Indeed, LKB1-deficient thymocytes have low Bcl-xL levels and are sensitive to apoptosis (Cao et al. 2010). Pradelli et al. showed that prolonged AMPK activation caused by inhibition of glycolysis suppresses mTOR signaling and leads to decreased translation of Mcl-1, sensitizing cells to apoptosis (Pradelli et al. 2010). Additionally, AMPK can phosphorylate and activate p53 after nutrient stress, with subsequent cell-cycle arrest and apoptosis (Jones et al. 2005; Okoshi et al. 2008).

Glucose deprivation also results in decreased flux through the hexoamine pathway that can lead to diminished protein glycosylation, protein misfolding, and ER stress. Protein glycosylation depends in part on *N*-acetylglucosamine (GlcNAc) and is important for proper protein folding in the ER (Kaufman et al. 2010). Misfolded proteins invoke the unfolded protein response (UPR) to reduce global protein synthesis and increase production of chaperone proteins to increase ER protein folding (Hoyer-Hansen and Jaattela 2007b). Initially, lack of glycosylation can greatly reduce glycosylation and surface expression of both nutrient transporters and growth factor receptors, which diminishes cell signaling to further exacerbate metabolic stress (Wellen et al. 2010). Ultimately, however, unresolved misfolded proteins lead to apoptotic death through transcription activation of proapoptotic proteins such as Bim and Puma and activation of apoptosis by the UPR-responsive transcription factor Chop/GADD153 (Oyadomari and Mori 2004; Ishihara et al. 2007; Puthalakath et al. 2007).

In some cases loss of glucose metabolism may lead to apoptosis even in cells lacking essential proapoptotic Bcl-2 family proteins. Two studies from Muñoz-Pinedo and colleagues showed that glucose deprivation could lead to apoptosis of Bak and Bax-deficient cells (Muñoz-Pinedo et al. 2003; Caro-Maldonado et al. 2010). In this case, Caspase 8 was activated to induce apoptosis. Surprisingly, however, Caspase 8 activation did not appear to be caused by death receptor signaling and was not Fas-dependent. Rather, Caspase 8 appeared to respond to metabolic stress and promote apopto-

sis through an unknown mechanism. Although poorly understood, this pathway may be critical as a link to promote the cell death of cancer cells that have acquired mutations to resist intrinsic pathways of apoptosis.

HYPOXIA AND CELL DEATH

Oxygen is essential to support mitochondrial metabolism, but ischemia, tissue damage, or poorly vascularized tumor microenvironments can limit oxygen distribution, leading to metabolic stress and potentially apoptosis (Melillo 2007; Rey and Semenza 2010). Under hypoxia, the normally short-lived transcription factors hypoxia-inducible factor 1/2 α (HIF1 α and HIF2 α) are stabilized to promote an adaptive response that increases anaerobic metabolic pathways. Regulation of HIF1/2 α by oxygen occurs through prolyl hydroxylation in an oxygen-dependent reaction that leads to degradation by the ubiquitin-ligase Von Hippel-Lindau (VHL) in normoxia. In hypoxia, prolyl hydroxylation is reduced and HIF1/2 α are stabilized (Rey and Semenza 2010). The HIF transcription factors promote a complex transcriptional program to increase anaerobic metabolic flux and survival by recruiting new vasculature and expressing Glut1 and glycolytic enzymes (Fulda and Debatin 2007). HIF also induces the small BH3-only protein BNIP3, which is not thought to induce apoptosis but rather promotes autophagic targeting of mitochondria, or mitophagy (Tracy et al. 2007; Burton and Gibson 2009). Interestingly, BNIP3 is frequently lost in cancer cells, suggesting that mitophagy may be an impediment to cancer cell response to hypoxia (Lee and Paik 2006).

Hypoxia and HIF1 α activation can also inhibit apoptosis on a transcriptional level. HIF1 α can up-regulate the antiapoptotic proteins Bcl-2, Bcl-xL, and Survivin and down-regulate the proapoptotic Bid and Bax (Erler et al. 2004; Liu et al. 2008b; Chen et al. 2009a,b). HIF1 α activity can also antagonize p53-mediated apoptosis, especially with combined DNA damage (Graeber et al. 1996; Hao et al. 2008; Sandoel et al. 2010). In some settings, however, HIF1 α has been found to stabilize p53, leading to

subsequent apoptosis (Suzuki et al. 2001; Hansson et al. 2002; Chen et al. 2003; An et al. 2004). The interaction between the HIF1 α and p53 may thus be highly context and cell-type dependent. More severe hypoxia or anoxia promotes apoptosis independent of the HIF1 α pathway. In these conditions, the electron transport chain collapses, expression of the antiapoptotic protein Mcl-1 is lost, and Bax and Bak are activated to induce apoptosis (Saikumar et al. 1998; Brunelle et al. 2007). Thus, changes in oxygen availability induce an adaptive response that may allow for extended survival, but severe or prolonged oxygen deprivation leads to apoptosis.

p53 IN METABOLIC STRESS

Metabolic stress also leads to p53 activation to induce apoptosis by up-regulating Puma, Noxa, and Bax (Nakano and Vousden 2001; Gottlieb et al. 2002; Rozan and El-Deiry 2007; Colloff et al. 2011). Lee et al. found that glucose deprivation caused the tricarboxylic acid (TCA)-cycle enzyme malate dehydrogenase (MDH) to bind to and activate acetylated p53, leading to cell-cycle arrest and apoptosis (Lee et al. 2009). AMPK activation on glucose withdrawal can also lead to p53 phosphorylation on serine 15 to cause cell-cycle arrest (Jones et al. 2005). This phosphorylation may also play a key role in cellular response to metabolic stress to induce Puma and apoptosis. It is unclear, however, if AMPK directly phosphorylates p53 or if an intermediate kinase is also involved.

Growth factor withdrawal also leads to p53 phosphorylation on serine 15 (18 in mouse) that is metabolically sensitive. We have observed Glut1/Hexokinase overexpressing cells that maintain growth factor-independent glucose metabolism can selectively suppress this phosphorylation and p53 activity after growth factor withdrawal (Mason et al. 2010). Importantly, changes in glucose metabolism also controlled p53 phosphorylation on treatment of BCR-Abl⁺ leukemic cells with imatinib mesylate, demonstrating that metabolic control of p53 activity may play a general role in the action of kinase inhibition in cancer therapy. This glucose-sensitive regulation of p53 occurred in part through

the activation of the lipid-sensitive kinase PKC δ and was independent from canonical DNA damage-induced p53 activation, which led to p53 activation independent of PKC δ and regardless of cellular metabolic state. Romero Rosales et al. (2009) also observed a proapoptotic role for PKC δ downstream from cytokine withdrawal. Importantly, PKC δ can confer sensitivity to apoptosis after chemotherapy or radiation treatment, and the loss of PKC δ has been associated with several kinds of aggressive cancer (Gonelli et al. 2009). Although it does not appear that PKC δ directly phosphorylates p53, this pathway nevertheless provides a metabolically sensitive mechanism for p53 regulation.

Acetylation may also link metabolism to p53 regulation. Acetyl-CoA is required for acetylation and is derived from the metabolism of glucose, the β -oxidation of long-chain fatty acids, or from the conversion of the TCA-cycle intermediate citrate via the ATP citrate lyase (ACL) enzyme (Salway 2004). Thus, levels of acetyl-CoA are sensitive to changes in growth factor input or nutrient availability. Indeed, Wellen et al. (2009) have recently shown that ACL is an important source of acetyl-CoA to support histone acetylation, and loss of growth factor input or glucose availability leads to a global decrease in acetyl-CoA and protein acetylation. p53 acetylation (Gu and Roeder 1997; Sakaguchi et al. 1998; Pearson et al. 2000) is critical for p53 to induce cell-cycle arrest or apoptosis (Lavin and Gueven 2006; Tang et al. 2008) and may be metabolically regulated. Indeed, we have showed that p53 is acetylated downstream from growth factor withdrawal in a metabolically sensitive manner, leading to a transcriptional program distinct from DNA damage-induced activation (Mason et al. 2010).

METABOLIC STRESS AND NECROSIS

Ultimately, necrosis may occur when cells do not meet their minimal bioenergetic demands (Jin et al. 2007). In these instances, a collapse of ATP levels may lead to failure of ATP-dependent sodium/potassium exchangers and osmotic stress and cell rupture. Necrosis may occur in metabolically challenging situations such

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as ischemia/reperfusion, in which tissue is subjected to nutrient deprivation during ischemia followed by an oxidative and ROS burst during reperfusion (Zong and Thompson 2006). Demonstrating the role of ATP in preventing necrosis, Leist et al. (1997, 1999) showed in seminal studies that T cells subjected to various forms of stress died by necrosis rather than apoptosis when ATP was depleted. To respond to metabolic stress, cells activate adaptive pathways to uptake or generate more nutrients, such as autophagy or the HIF pathway. Thus, inhibiting autophagy in the presence of metabolic stress has been shown to lead to metabolic catastrophe and necrosis (Lum et al. 2005; Degenhardt et al. 2006), and a similar effect has been observed in hypoxic cells deprived of proper HIF signaling (Tennant et al. 2009).

Some cell stresses may lead to artificial nutrient depletion that can cause necrosis. In particular, DNA repair performed by poly (ADP-ribose) polymerase (PARP) can also cause necrosis if PARP activity is excessive. PARP requires cytosolic nicotinamide adenine dinucleotide (NAD^+) as a substrate to repair various kinds of DNA damage including single- and double-strand breaks and base excision repair (Sodhi et al. 2010). If DNA damage is extensive, PARP can deplete NAD^+ , causing a collapse of glycolysis, loss of ATP production, and subsequent necrosis (Martin et al. 2000; Cipriani et al. 2005). Consistent with this ability of PARP to lead to necrosis, caspase-dependent cleavage of PARP may be a key step to prevent energy depletion and allow apoptosis to occur (Sodhi et al. 2010). Although metabolic collapse and necrosis would seem to be an attractive strategy in which to target cancer cells, excessive necrosis has been shown to lead to inflammation and promotion of advanced tumorigenesis (Balkwill et al. 2005), so treatment strategies that instead cause apoptotic death are often pursued.

CONCLUDING REMARKS

Mammalian cells rely on extracellular nutrient uptake to maintain metabolism and provide bioenergetic precursors for macromolecular

synthesis. This uptake is normally under tight control of growth factors, but oncogenes and oncogenic kinases can mimic growth factor signaling to promote cell intrinsic nutrient uptake and metabolism. In particular, activation of Akt can promote glucose, amino acid, and lipid uptake (Edinger and Thompson 2002; Wieman et al. 2007). Nutrient withdrawal, either by direct means, growth factor deprivation, or oncogenic kinase inhibition, leads to metabolic stress and a set of responses that may ultimately result in cell death, and understanding these responses may be critical in efforts to exploit cancer metabolism. It is now clear that autophagy plays a key role to reduce intracellular stress and provide nutrients to replace diminished extracellular nutrient uptake, and inhibition of autophagy can enhance the proapoptotic ability of oncogenic kinase inhibitors (Kamitsui et al. 2008; Bellodi et al. 2009; Altman et al. 2011; Guo et al. 2011). Continued nutrient deprivation will regulate Bcl-2 family proteins to induce Puma and Bim and activate Noxa while down-regulating Mcl-1 to promote apoptosis. If cells resist apoptosis or if nutrient deprivation is severe, necrosis can occur. Ultimately, understanding links between nutrient stress, autophagy, and cell death through apoptosis or necrosis will be central to inhibiting cancer cell metabolism in novel metabolic cancer therapies.

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