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Regulators mount up: the metabolic roles of apoptotic proteins

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The induction of apoptosis, a programmed cell death pathway governed by activation of caspases, can result in fundamental changes in metabolism that either facilitate or restrict the execution of cell death. In addition, metabolic adaptations can significantly impact whether cells in fact initiate the apoptotic cascade. In this mini-review, we will highlight and discuss the interconnectedness of apoptotic regulation and metabolic alterations, two biological outcomes whose regulators are intertwined.

KEYWORDS

apoptosis, metabolism, caspases, mitochondria, MOMP

Introduction

Apoptosis is a programmed cellular suicide mechanism with important roles in the maintenance of multicellular organisms. The classical intrinsic apoptotic pathway begins with an apoptotic stimulus that alters pro-apoptotic proteins at the mitochondria. Subsequently, this leads to outer membrane permeabilization (MOMP) as a consequence of Bax/Bak oligomerization (Oltval et al., 1993; Shi et al., 2003; Buytaert et al., 2006; McArthur et al., 2018). MOMP allows for the secretion of soluble cytochrome *c* into the cytosol where it binds to apoptotic protease activating factor 1 (Apaf-1) and causes the formation of the apoptosome (Schafer and Kornbluth, 2006; Riedl and Salvesen, 2007). Apoptosome formation triggers the concomitant cleavage and activation of caspase 9, which cleaves and activates caspases 3 and 7 ultimately culminating in the death of the cell (Xue and Robert Horvitz, 1995; Elmore, 2007; McIlwain et al., 2013). Each of the proteins listed are critically important for proper execution of the intrinsic apoptosis pathway. However, these proteins (and others known to regulate apoptosis) do not solely function as determinants of whether a cell lives or dies. Rather, many of the main players in apoptotic pathways have other, oftentimes vital, roles in cellular processes such as metabolism. Perhaps the best example of the dual role of certain apoptotic proteins within metabolic pathways is the aforementioned cytochrome *c*, whose apoptotic role was discovered in the laboratory of Xiaodong Wang (Liu et al., 1996; Li et al., 1997). While the discovery of a pro-apoptotic role for a protein best understood to function in the mitochondrial respiratory chain was (at the time) surprising, the capacity of cytosolic cytochrome *c* to activate caspases is now central to our understanding of programmed cell death. In this article, it is our objective to highlight the sometimes-underappreciated metabolic functions of apoptotic regulators and to discuss the circumstances in which metabolism directly impinges upon the execution of apoptosis.

Glucose flux and the capacity of p53 to induce apoptosis

As mentioned above, turnover of aged or defective cells in the human body can often occur through the induction of apoptosis (Kerr et al., 1972; Fawthrop et al., 1991). The

execution of apoptosis is tightly regulated by a number highly conserved and distinct signal transduction networks. In addition, proteins which regulate apoptotic death can be broadly separated into two main categories: pro-apoptotic and anti-apoptotic. Anti-apoptotic proteins function to provide defenses against the activation of proteases that execute the apoptotic program. Meanwhile, pro-apoptotic effectors can sense deleterious events in the cell and sound the alarm to initiate the induction of apoptosis. Alternatively, pro-apoptotic effectors can function directly in the cleavage of target proteins that ultimately contribute to the orderly dismantling of the cell. Dysregulation of these coordinated pathways is linked to pathological conditions like fibrosis, autoimmune disease, neurodegeneration, and cancer (Elmore, 2007).

One of the most well-studied pro-apoptotic regulators is p53, a known tumor suppressor that integrates extracellular and intracellular signals to promote cell death (Surget et al., 2013; Aubrey et al., 2018). The best characterized activator of p53 is DNA damage, which ultimately causes phosphorylation and stabilization of the p53 protein and thus allows it to transcriptionally activate downstream targets. However, nutrient availability and energy demands can also impact p53 activity (Horn and Vousden, 2007). Previous work has demonstrated that high levels of ADP promote the binding of p53 to DNA whereas elevated ATP levels block this interaction (Okorokov and Milner, 1999). There are also significant links between the withdrawal of growth factors, changes in nutrient uptake and p53. For example, serum starvation can often promote p53-mediated increased apoptosis due to elevated expression of p53 upregulated modulator of apoptosis (PUMA) (Ekoff et al., 2007). In contrast, if glucose uptake is maintained (e.g., through localization of Glut1 to the plasma membrane) after growth factor withdrawal, apoptosis is attenuated as a consequence of glycolytic flux-mediated inhibition of p53 and PUMA (Zhao et al., 2008).

In addition to the links between glucose metabolism and PUMA, the withdrawal of extracellular glucose from cells acts as a trigger for the nucleocytoplasmic isoform of the metabolic enzyme malate dehydrogenase (Lee et al., 2009). This enzyme is normally involved in the malate-aspartate shuttle, and can function to stabilize and transactivate p53 to promote p53-dependent cell-cycle arrest. Similarly, withdrawal of glucose often leads to the activation of the AMP-activating protein kinase (AMPK), a cell cycle arrest at the G (1)/S stage, and the induction of p53 (Jones et al., 2005; Okoshi et al., 2008).

Oftentimes, the regulation of apoptosis by p53 is largely a consequence of post-translational modifications to key residues that affect protein stability and ultimately the genes targeted by this transcription factor. Indeed, the AMPK-mediated p53 activation during glucose deprivation is dependent upon the phosphorylation of serine 15 (Jones et al., 2005). Similarly, the capacity of glucose to prevent p53 induction of PUMA depends on a decrease in the phosphorylation of serine 46 (Garufi and D'Orazi, 2014). In addition to these roles, the decrease of glucose utilization can lower the apoptotic threshold of cells due to alterations in the cytoplasmic function of p53. A good example of this p53-mediated effect can be found in patient-derived models of glioblastoma which have been pharmacologically restricted in their glucose uptake. These tumors are primed for apoptosis *in vivo* but are kept alive

owing to the sequestration of cytoplasmic p53 by Bcl-xL and the prevention of p53 mediated transcription of pro-apoptotic factors keeping the tumors just below the threshold of cell death (Mai et al., 2017). Lastly, p53 can function to ensure that elevated glucose flux cannot prevent the initiation of the apoptotic cascade by inhibiting the expression of the glucose transporters Glut1 and Glut4 (Schwartzberg-Bar-Yoseph et al., 2004).

TIGAR: a protein that functions downstream of p53 to impact metabolism

The understanding of how metabolic flux impacts p53 regulation of apoptosis also inspired efforts to study downstream effectors of p53 that impact metabolism. One particular example of p53-mediated metabolic regulators is TP53-inducible glycolysis and apoptosis regulator (TIGAR). TIGAR was discovered through microarray analysis of genes following p53 induction and functions to attenuate glycolytic flux through regulation of fructose-2,6-bisphosphatase (Bensaad et al., 2006). The capacity to regulate fructose-2,6-bisphosphatase is dependent on TIGAR's phosphatase activity, which inhibits the flow of carbon units through glycolysis and prevents glycolysis from counteracting the action of p53. In addition, the activity of TIGAR not only blocks glycolysis, but also can promote cell survival by diverting glycolytic flux into biosynthetic or of antioxidant-generating pathways. One key determinant of TIGAR function is subcellular localization, which is highly dependent on various stress stimuli encountered by the cell. While in the cytoplasm, TIGAR antagonizes glycolysis and shifts carbon flux into the pentose phosphate pathway (PPP), which promotes production of NADPH for redox homeostasis or nucleotide synthesis. However, exposure to DNA damaging chemotherapeutic agents causes TIGAR to translocate to the nucleus where it halts the cell cycle and promotes DNA damage repair in a p53-dependent manner (Yu et al., 2015). Hypoxia can also impact the subcellular localization of TIGAR. Under hypoxic conditions, TIGAR can localize to the outer mitochondrial membrane where it binds to hexokinase 2 (HK2) and increases HK2 activity. The binding of TIGAR to HK2 increases glycolytic flux and helps to lower mitochondrial reactive oxygen species (ROS) with TIGAR functioning as a scaffold for HK2 activation (Cheung et al., 2012). Studies have also examined the loss of TIGAR, which can promote glucose oxidation and glycolysis in myocardial tissue demonstrating the importance this enzyme plays in throttling glycolytic flux following p53 activation (Okawa et al., 2019). Taking all of this together, it is clear that TIGAR is an important link between p53 and glycolytic metabolism. However, p53 induced genes can reciprocally counteract this effect to facilitate p53-mediated apoptosis. Thus, p53 exemplifies a "push-pull" dynamic between one of the best studied tumor suppressors (p53) and a common metabolic alteration observed in cancer cells (increased glycolysis) with important ramifications for disease progression.

MOMP and metabolism

Following the initiation of apoptotic signaling, pores are formed in the outer mitochondrial membrane during MOMP to allow

proteins from the mitochondrial intermembrane space, including cytochrome *c*, to reach the cytosol and propagate the apoptotic cascade (Westphal et al., 2011). Although cytochrome *c* release was once considered the point of no return for cell death, research over the past decade indicates that sub-lethal amounts of MOMP may occur in response to certain apoptotic stimuli. This phenomenon is known as incomplete or “minority” MOMP (Tait et al., 2010; Ichim et al., 2015). For a cell to survive MOMP it must either limit the formation of the pore, neutralize the cytochrome *c* that is released, or functionally adapt to improve its fitness in this environment.

Blocking formation of the pore in the mitochondrial membrane is a logical place for the beginning of our discussion on links between metabolism and MOMP. Indeed, bioactive lipids, such as sphingolipids, have been implicated as a significant player in the regulation of MOMP in a variety of cell types. More specifically, ceramide, a class of sphingolipid, can induce apoptosis through a mechanism that is dependent on the molecular machinery required to promote MOMP (Taha et al., 2006; von Haefen et al., 2002). Furthermore, inhibition of sphingolipid metabolism through the use of pharmacological inhibitors is capable of preventing MOMP as it blocks the interaction between ceramide metabolites and inducers of MOMP (Chipuk et al., 2012). Relatedly, cancer cells can manipulate sphingolipid metabolism to decrease the intracellular levels of ceramide to maintain mitochondrial membrane integrity. As an example, sphingomyelin synthases are activated in leukemic cells to decrease the levels of ceramide and prevent stress-induced apoptosis (Dolgachev et al., 2004; Separovic et al., 2007; Lafont et al., 2010).

However, if a cell cannot alter metabolism to prevent MOMP, another strategy to survive involves the neutralization of the cytochrome *c* that is released from the inner membrane space. One such strategy to neutralize the execution of apoptosis employed by these cells is the rewiring of metabolism to defang cytosolic cytochrome *c* and conserve viability. Retroviral cDNA screens following MOMP have shown a role for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in promoting survival through increased glycolytic flux in the cell when caspase activation was inhibited (Colell et al., 2007). Interestingly, cytochrome *c* released from these cells following MOMP was re-localized back to the mitochondria only in cells with increased GAPDH expression. These interesting results indicate that GAPDH can promote recovery and function of those mitochondria post-MOMP. In addition, the pro-apoptotic activity of cytochrome *c* relies on its redox state and is known to be controlled by ROS signaling. Increased glucose flux into the PPP of cancer cells generates elevated levels of the antioxidant glutathione, which can also function to antagonize cell death by numerous mechanisms [e.g., ferroptosis (Dixon et al., 2012)]. However, with regards to apoptotic regulation, glutathione can inactivate cytochrome *c* following MOMP to prevent apoptosis (Vaughn and Deshmukh, 2008). Instead of attempting to inactivate cytochrome *c*, a cell can also survive during MOMP by simply eliminating the cytochrome *c* that is released. RNAi screens in both neurons and cancer cells uncovered a conserved strategy for dealing with cytoplasmic cytochrome *c*. p53-associated Parkin-like cytoplasmic protein (PARC) functions as an E3 ligase that targets cytochrome *c* for destruction following mitochondrial stress and minority MOMP to promote viability (Gama et al., 2014). In some cases, adaptation to a sub-lethal MOMP benefits a cell and may

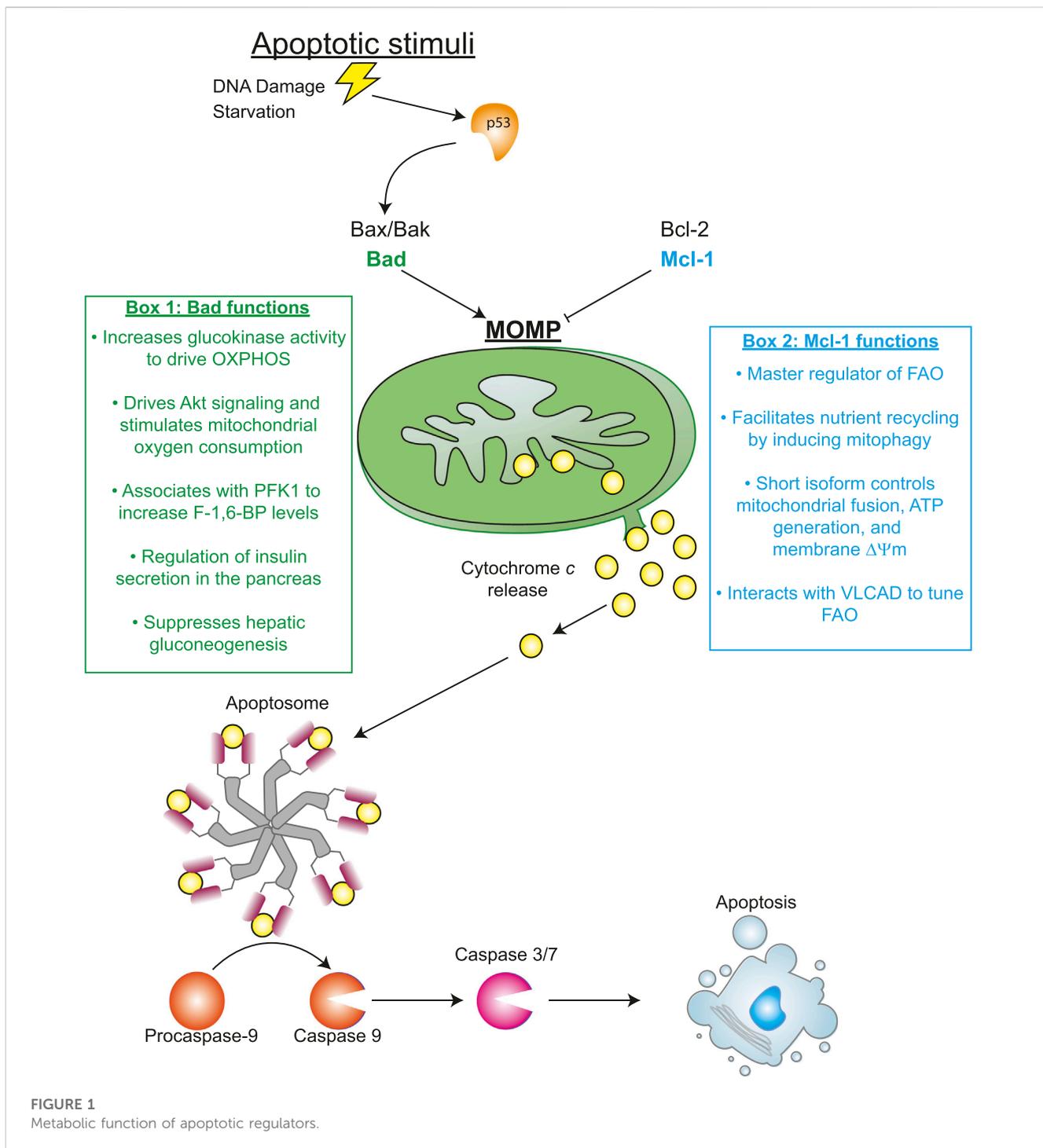
eventually promote tumorigenesis. Due to the fact that minority MOMP induces only limited caspase activation to cause DNA damage, this can contribute to genomic instability and result in oncogenic transformation (Ichim et al., 2015). Malignant cells that have been treated with agents to induce MOMP allows for cytosolic cytochrome *c* to activate the heme-regulated inhibitor kinase (HRI) engaging the integrated stress response (ISR) (Kalkavan et al., 2022). The engagement of the ISR by cancerous cells generates a drug-resistant cell population that is not only protected against apoptosis but also abrogates the efficacy of the therapy. Therefore, minority MOMP can not only promote tumorigenesis but also contribute to therapeutic resistance in malignant cells.

Metabolic functions of Bcl-2 family members

The formation of pores in the outer mitochondrial membrane as a consequence of the regulation of Bcl-2 family members is often critical to the induction of apoptosis. As discussed above, the Bcl-2 proteins have multiple structural and functional similarities with the ability to be either pro- or anti-apoptotic. For example, Bcl-2 family members are well known to contain BH3 motifs (Bcl-2 homology 3) (Blaineau and Aouacheria, 2009), which have diverse biological functions in the regulation of apoptosis. However, many of the Bcl-2 family members also have a significant role in the regulation of metabolism. Notably, while Bcl-2 family members have been shown to regulate mitochondrial dynamics and thereby indirectly alter metabolic pathway utilization (Autret and Martin, 2010), we have chosen to focus the section below on the involvement of Bcl-2 family members on metabolic flux directly.

One such Bcl-2 family member with a significant metabolic function is Myeloid cell leukemia-1 (MCL1 or Mcl-1) which localizes to the mitochondria to prevent MOMP and blocks apoptosis (Kozopas et al., 1993; Reynolds et al., 1994; Zhou et al., 1997; Opferman et al., 2003; Opferman et al., 2005). Mcl-1 is among the most overexpressed survival proteins across all human cancers and is linked to poor survival and therapeutic resistance (Wuillème-Toumi et al., 2005; Wei et al., 2006; Beroukhim et al., 2010). In addition to its anti-apoptotic function, Mcl-1 has been shown to facilitate nutrient recycling through the induction of mitophagy. Although Mcl-1 inhibits nonselective autophagy caused by nutrient starvation, Mcl-1 promotes the targeted destruction of depolarized mitochondria ensuring a functional mitochondrial pool to meet energetic needs (Moyzis et al., 2022). However, increased mitophagy is not always beneficial to the cell. Recent investigations have suggested that overactive mitophagy may prove detrimental to the cell and compromise viability depending on the context of the cellular stressor (Hawk et al., 2018). One can therefore postulate that perhaps a sustained increase in Mcl-1 activity would deplete the mitochondrial pool below required levels.

Mcl-1 also helps cancerous cells meet energetic needs through direct regulation of metabolic pathways (see Box 2 in Figure 1). These functions can be tied to alternative splicing as distinct Mcl-1 spliceoforms can impact metabolism in unique manners. The full-length isoform associates with the outer mitochondrial membrane where it regulates cell death. However, the short isoform of Mcl-1 is imported into the inner mitochondrial matrix where it regulates



fusion, ATP production, membrane potential, and maintenance of ATP synthase to support the adenylate energy charge of the cell (Perciavalle et al., 2012). The short isoform of Mcl-1 also has a direct impact on fatty acid oxidation (FAO). The α helix of Mcl-1 directly interacts with very long-chain acyl-CoA dehydrogenase (VLCAD) in nutrient deprived conditions to dynamically tune FAO (Escudero et al., 2018). This metabolic action of Mcl-1 during periods of stress may provide additional support to cancer cells that extend beyond survival. In fact, in B Cell acute lymphoblastic leukemias (B-ALL),

where Mcl-1 overexpression is a defining characteristic, FAO is a critical fuel source. Expression of Mcl-1 correlates with elevated FAO gene signatures in these malignancies and loss of Mcl-1 rewires fuel utilization from catabolism of fatty acids to a reliance upon glycolysis (Prew et al., 2022). As such, in the absence of apoptotic stimuli, Mcl-1 can function as a significant regulator of the FAO program.

Pro-apoptotic Bcl-2 family members also have well-defined functions in metabolic pathways. For example, Bcl-2 associated

agonist of cell death (BAD) promotes programmed cell death by binding to Bcl-2 and preventing its ability to block MOMP and cytochrome *c* release (Yang et al., 1995). BAD is often regulated by phosphorylation which determines not only its role in apoptosis, but also the specific function it plays in metabolism (See Box 1 in Figure 1). For example, BAD affects glucose utilization by forming a complex containing glucokinase at the mitochondria due to its phosphorylation at S112. As a consequence, the activity of glucokinase is increased in a fashion that drives mitochondrial oxidative phosphorylation (Danial et al., 2003). Meanwhile, phosphorylation of BAD at S118 and S99 has been shown to affect metabolism due to altered Akt signaling, stimulation of complex I and elevated mitochondrial oxygen consumption (Mann et al., 2019). BAD can also bind to other metabolic enzymes as immunoprecipitation experiments have demonstrated that it can associate with phosphofructokinase 1 (PFK1). Functional assays demonstrate that JNK1-mediated phosphorylation of BAD at T201 increases PFK1 function to raise fructose-1,6-bisphosphate generation, a key rate-limiting step of glycolysis (Deng et al., 2008). Some of the post-translational modifications to BAD are cell type-specific for distinct tissue metabolic functions. For example, modification to the S155 site in the BH3 domain of BAD allows the protein to control glucose stimulated insulin secretion in the beta cells of the pancreas (Danial et al., 2008). Like the pancreas, liver-specific activity of BAD modulates the metabolism of hepatic cells. The liver must precisely balance gluconeogenesis, the generation of glucose from other metabolic substrates, and glycolysis to maintain homeostasis. BAD maintains this physiological balance by employing phosphorylation of its BH3 domain as a sensor to activate glucokinase and suppress gluconeogenesis to properly coordinate hepatic glucose output (Giménez-Cassina et al., 2014).

Links between dietary metabolism, apoptosis, and disease

The interplay between metabolism and apoptosis extends beyond the individual cell as whole-organism metabolism impacts death across tissue systems. For example, it has long been known that heightened availability of the sugar glucose is beneficial to tumorigenesis and cancer cell survival (Warburg, 1956; Liberti and Locasale, 2016). In fact, the metabolic rewiring of cells to promote increased glucose uptake and glycolytic flux is a hallmark of cancer (Hanahan and Weinberg, 2011). Monosaccharides in general are not universal in their impact on cell survival *versus* death, and thus, the specific form of sugar taken up by cells could alter the balance between tumor promotion and tumor suppression.

In addition to glucose, fructose has also been shown to promote cancer through inhibition of apoptotic pathways. Nutrients taken in through diet enter the tissue of mammals at the epithelium of the small intestine and colon. In the small intestine, sugars from the diet (e.g., glucose and fructose) enter the cell through the Glut family of transporters where they are phosphorylated by hexokinase (HK) and ketohexokinase (KHK) respectively (Miller et al., 1956; Roberts and Miyamoto, 2015; Jang et al., 2018). The uptake of these metabolites is governed by the villus structure within the small intestine. Intestinal villus length is balanced by proliferation and death of intestinal epithelial cells (IECs) (Hall et al., 1994). The migration of IECs up

the villus separates them from their blood supply leading to hypoxia which ultimately can cause their apoptotic death. However, fructose increases the survival of hypoxic cells as well as IECs of the small intestine in mice (Taylor et al., 2021). Fructose in the IECs results in the accumulation of fructose-1-phosphate which competes with fructose-1,6-bisphosphate for the binding pocket of the glycolytic enzyme pyruvate kinase (PKM2). In this setting, inhibition of glycolytic flux, through decreased PKM2 activity, allows for increased upstream glycolytic metabolites to counteract the hypoxic insult and thereby dampen the apoptotic stimulus (Luo et al., 2011; Taylor et al., 2021). The cancer-causing potential of fructose is solidified by the finding that excessive levels of fructose in the colon, owing to the consumption of high fructose corn syrup, increases tumorigenesis in murine models (Goncalves et al., 2019). Importantly in this study, fructose was found to saturate the Glut receptors on the IECs causing fructose levels to still be high even into the colon where it could exert its neoplastic potential. Both glucose and fructose benefit malignant cells by promoting growth while simultaneously blunting apoptotic signaling.

Alternatively, other sugar molecules—including those deemed “rare” sugars—are deleterious to cancer cells. Rare monosaccharides with structural similarity to glucose or fructose induce apoptosis of cancer cells and may function as tumor suppressors. One such rare sugar, D-allose, has been shown in human head and neck cancers to induce apoptosis due to competitive inhibition of glucose uptake and lowering of the apoptotic threshold to improve efficacy of the taxane docetaxel (Mitani et al., 2009; Indo et al., 2014). Another rare sugar, L-sorbose, the C-3 epimer of fructose, is taken up and initially phosphorylated similarly to fructose by KHK to produce L-sorbose-1-phosphate (S-1-P). However, unlike fructose, S-1-P inhibits the action of HK at the top of glycolytic flux thereby preventing the synthesis of essential glycolytic metabolites and promoting apoptosis (Xu et al., 2023). In this same vein, mannose, the C-2 epimer of glucose, is also tumor suppressive. Mannose enters the cell through Glut transporters and is phosphorylated by HK to mannose-6-phosphate (M6P). M6P decreases the levels of the anti-apoptotic proteins Mcl-1 and Bcl-X_L by inhibiting key glycolytic enzymes including HK and sensitizing cells to the chemotherapeutic cisplatin (Gonzalez et al., 2018). These findings importantly show that it is not necessarily the abundance of sugar but rather the specific sugar source which tips the balance between tumorigenesis and cell death.

Conclusion and perspectives

As discussed here, apoptosis and metabolism are tightly interwoven cellular processes. The choice of whether or not to activate the cellular suicide program can be dictated by nutrient availability and metabolic pathway engagement. Apoptosis is kept in check through the sequestration of important apoptotic players (e.g., cytochrome *c* in the mitochondria) during normal metabolic flux with robust nutrient sources. Members of the Bcl-2 family of proteins play integral roles in properly trafficking nutrients through their requisite pathways to ensure proper growth and division of the cell. Apoptosis-inducing stimuli alter metabolic flux modifying the function, localization, or both of apoptotic proteins which then either must be blocked or the cell will succumb to this insult. There is inherent difficulty in decoupling the metabolic and

death role of apoptotic proteins. The examination of the metabolic role of these proteins through loss of function studies can prove lethal to the cell. Therefore, thorough consideration must be taken to safeguard cell viability when attempting to disentangle the metabolic role from the cell death aspect. Mutations in these proteins which specifically inactivate the apoptotic domain (e.g., altering the BH3 domain of Bcl-2 family proteins) are a useful tool for elucidating the metabolic pathways regulated by the protein of interest. Likewise, in basal conditions or those that do not meet the apoptotic threshold, such as minority MOMP, the metabolic impact of apoptotic regulators could better be considered. This is particularly important given the prevalence of chemotherapeutics which specifically target Bcl-2 family members to kill cancer cells while simultaneously having a metabolic impact on non-malignant tissues (such as immune cells, cancer-associated fibroblasts, etc.). On the other hand, the advent of recently approved cancer therapeutics targeting metabolism may inadvertently trigger pathways that regulate apoptosis (Stine et al., 2022). These alterations to apoptotic signaling may be synergistic or may function to counteract the efficacy of these drugs, but possible knock-on impacts to apoptosis must be considered. Ideally, future ventures to treat cancer will exploit the relationship between apoptosis and metabolism to simultaneously activate the cellular death programs while starving transformed cells of nutrients required for survival.

Author contributions

JS and ZS jointly conceptualized, drafted, and edited this contribution. All authors contributed to the article and approved the submitted version.

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