The role of metabolic checkpoint regulators in B cell survival and transformation

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Abstract
In response to mitogenic stimulation, B cells activate different pro-anabolic signaling pathways such as c-Myc- and mTORC1-dependent networks to satisfy the energetic demands of biomass synthesis and proliferation. In order to preserve viability and function, cell growth cannot progress unchecked and must be adjusted according to the availability of nutrients. Nutrient-sensing proteins such as AMPK antagonize mTORC1 activity in response to starvation. If pro-anabolic signaling pathways are aberrantly activated, B cells may lack the metabolic capacity to accommodate their energetic needs, which can lead to cell death. On the other hand, metabolic hyperactivation is a salient feature of cancer cells, suggesting that mechanisms exist, which allow B cells to cope with metabolic stress. The aim of this review is to discuss how B cells respond to a mismatch between energy supply and demand and what the consequences are of metabolic dysregulation in normal and malignant B cells.

Keywords
anabolism, autophagy, B cells, metabolic stress, mTORC1, senescence

1 | INTRODUCTION

B cells contribute substantially to protection against pathogens. Their ability to produce pathogen-specific antibodies, to present antigen to T cells, and to regulate other cells through the secretion of cytokines makes them major players of our adaptive immune response.¹,² On the flip side, B cells are also known to play pathologic roles in a number of diseases including autoimmune disorders³,⁴ and various malignancies.⁵ Throughout their life, B cells assume different roles and are exposed to changing environments, which is reflected by dynamic adjustments of their gene expression profile and their metabolic signature.⁶,⁷ B cell development and differentiation are accompanied by transient switching between fairly quiescent stages and stages characterized by rapid proliferation or increased protein secretion. Nutrient uptake and the activity of the main metabolic pathways need to be quickly adjusted to meet the specific metabolic demands of any given B cell subset. Much of the current research in B cell metabolism is focused on elucidating the molecular mechanisms driving these metabolic adaptations.⁷,⁸ Growth factor–mediated signaling induces the expression of different nutrient transporters, metabolic enzymes, and other components of the metabolic machinery to supply growing cells with energy and biosynthetic precursor molecules. Similarly, the function of B cell subsets displaying high rates of protein secretion such as the B1 cells and plasma cells is supported by their specific metabolic profile.⁹,¹⁰ While significant progress has been made toward a better understanding of the molecular mechanisms driving anabolic metabolism and cell growth in B cells,⁷,⁸ it is less well understood how B cells return to a state of quiescence.
and what the consequences are if they fail to do so. Metabolic regulators must exist, which help to maintain metabolic quiescence in naive B cells, which ensure that energy consumption does not exceed available resources in proliferating B cells and which return B cells to a resting state upon cell cycle exit. The consequences of inappropriate activation or inhibition of these metabolic checkpoints regulators on B cell fate and function are however difficult to predict. A mismatch between energy consumption and generation can result in metabolic stress and ultimately energetic collapse. Hence, hyperactivation of anabolic metabolism due to a deletion or inhibition of metabolic regulators can have a negative impact on B cell viability. Yet despite aberrant activation of signaling molecules that drive hyperactivation of anabolic processes, malignant B cells are able to survive. This suggests that adaptive mechanisms exist that can help B cells to cope with metabolic stress. The aim of this review is to summarize the current knowledge on metabolic checkpoint regulators in B cells and to discuss the implications of dysregulated metabolic homeostasis for the survival of normal and malignant B cells.

2 | METABOLIC BALANCE IN B CELL PRECURSORS

B cells are generated from hematopoietic stem cells in the bone marrow through a coordinated series of lineage-commitment steps governed by cytokines and other developmental cues. While progressing through the intermediate stages of this process, B cell precursors undergo sequential rearrangement of their immunoglobulin loci. First, the diversity (D) and joining (J) segments of the immunoglobulin heavy chain locus are recombined in pre- and pro-B cells. Subsequently, variable (V) gene segments are recombined to the rearranged D-J regions in pro-B cells. If a heavy chain is successfully produced, it is paired with the proteins of the surrogate light chain and the signaling subunits Igα and Igζ to form the pre-B cell receptor (pre-BCR). The pre-BCR promotes the proliferative expansion of the population of large pre-B cells. After the cells cease to proliferate, they rearrange the immunoglobulin light chain locus and assemble the B cell antigen receptor (BCR). Upon successful expression of a signaling competent BCR, immature B cells migrate to the spleen and finish maturation. B cell development is accompanied by phases of active proliferation and stages of cellular quiescence. Pro-B cells proliferate in response to interleukin-7 (IL-7), a cytokine produced by the bone marrow stromal cells. Large pre-B cells proliferate upon pre-BCR expression but remain dependent on IL-7 for their survival. To progress in their development, large pre-B cells need to arrest proliferation. Consistent with their different rates of proliferation, pro-B cells, large and small pre-B cells, and immature B cells differ significantly in their metabolic profile. To foster cell growth, IL-7 induces activation of signaling molecules such as mechanistic target of rapamycin complex 1 (mTORC1) and c-Myc, which drive an anabolic metabolic program indispensable for B cell development (Figure 1). Both mTORC1 and c-Myc are known metabolic master regulators, which are activated in response to mitogenic signals. mTORC1 phosphorylates downstream targets promoting protein synthesis, oxidative metabolism, and glycolysis. Myc is a transcription factor and drives the expression of genes involved in nutrient uptake, mitochondrial function, glycolysis, and glutaminolysis. While it may not be surprising that c-Myc/mTORC1-driven nutrient uptake and energy generation are essential for the proliferation of B cell precursors, the involvement of these pathways in regulating B cell metabolism prompts the question what effects would unrestrained mTORC1/c-Myc activity have on B cell development. Would mTORC1-driven hypermetabolism promote B cell expansion and render the cells vulnerable to malignant transformation? Or would unrestrained metabolic activity lead to an energetic crisis and a metabolic collapse? Recent studies suggest that developing B cells are particularly sensitive to a mismatch between energy consumption and nutrient availability. In normal cells, mTORC1 is inhibited upon starvation to restrict energy-consuming processes such as protein biosynthesis. An important inhibitor of mTORC1 activity in response to reduced energy levels is the enzyme AMP-activated protein kinase (AMPK; Figure 1). The deletion of the AMPK activator liver kinase B1 (LKB1) has been shown to result in a complete block in B cell development in Fnip1-deficient mice suggesting that B cell loss is not caused by a missing pre-BCR signal or a failure to induce the recombination of the light chain locus in this mouse model. Rather than playing a role in pre-BCR signaling, Fnip1 seems to be required to inhibit cell growth and metabolic activity when energy and nutrient consumption exceed the availability of the necessary resources. Fnip1-deficient pre-B cells show increased AMPK activation, but fail to efficiently terminate mTORC1 signaling in response to the chemical AMPK activator 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR). In comparison with wildtype cells, Fnip1−/− pre-B cells display enhanced glucose uptake, increased mitochondrial mass and a hypermetabolic phenotype but reduced ATP levels in response to IL-7 stimulation. In vitro, Fnip1−/− pre-B cells are able to progress in their development to IgM positive cells; however, the generation or survival of these cells is strongly dependent on high levels of amino acids. Collectively, these studies suggest that the maintenance of metabolic homeostasis is crucial for B cell development and an inability to limit anabolic processes results in cell death rather than excessive proliferation.

3 | METABOLIC HOMEOSTASIS IN B CELL-DERIVED LEUKEMIA

The dramatic loss of cell viability in response to aberrantly increased metabolic demands in B cell precursors suggests that metabolic checkpoints are essential for B cell development. Intriguingly, not all B cell subsets require LBK1/AMPK activity for their survival or
proliferation. While a disruption of AMPK signaling results in increased apoptosis of developing B cells, mature stimulated B cells are capable of proliferation in the absence of LBK1 or AMPK activity. This suggests that these metabolic regulators are not essential for B cell proliferation in general. This prompts the question: What is the evolutionary benefit of such a high sensitivity to metabolic dysregulation in B cell precursors? From the work of Markus Müschen, a compelling model has emerged, in which metabolic checkpoints represent safeguard mechanisms against malignant transformation or autoreactivity. B cell precursors are at particular risk for malignant transformation due to their active DNA rearrangement and high levels of proliferation. Therefore, mechanisms mitigating the risk of malignant transformation are crucial at this stage.

Cancer cells are characterized by rapid proliferation associated with increased metabolic activity. Signaling pathways driving anabolic metabolism are frequently hyperactivated in cancer. Enhanced mTORC1 activity, and consequently increased metabolism, may represent a warning sign for B cells that transforming events have occurred. Several studies suggest that an inability to curtail mTORC1-signaling does not only inhibit B cell development but also limit the survival of malignant B cells. BCR-Abl1-transformed pre-B cells have been shown to lose viability upon the deletion of Lkb1 or Agrp.
defect in B cell development, Fnip1 deficiency prevents lymphoma development in the Eµ-Myc model. Myc is an oncogene known to reprogram cellular metabolism to sustain the high rate of proliferation in tumor cells. In normal cells, c-Myc expression is induced upon mitogenic stimulation. Additionally, c-Myc expression and function are also dependent on nutrient availability. While c-Myc expression escapes growth factor control in transformed cells, there is little evidence that metabolic control of Myc function is disrupted as well. It is tempting to speculate that the inability of Myc transgenic cancer cells to grow in the absence of Fnip1 shows that unrestrained metabolic activity is a liability rather than an advantage for cancer cells.

Nevertheless, it remains incompletely understood which metabolic checkpoint regulators are crucial for the survival of B cell–derived lymphoma or leukemia. While Fnip1 deficiency is incompatible with normal B cell development and lymphoma progression, the deletion of Ampkα1 accelerates lymphoma progression in the Eµ-Myc mouse model. Thus, the exact contribution of AMPK1, mTORC1 and Fnip1 to the metabolic homeostasis in normal and transformed B cell precursors remains to be determined.

Alongside mTORC1, the PI3K signaling pathway is also frequently hyperactivated in cancer cells and drives cell metabolism and proliferation. The inhibition of PI3K signaling in

**FIGURE 2** The impact of metabolic dysregulation on the survival and proliferation of different B cell subsets. Molecules that regulate metabolic checkpoints at different developmental stages are shown. An arrow signifies the depicted molecule plays a positive role in cell survival or proliferation. Negative regulation is marked by a line with a bar at the end.
normal B cell development is needed in order to release the transcription factor forkhead box protein O1 (Foxo1) from inactivation. Foxo1 activity is essential for the expression of IL7-receptor and the Rag enzymes, which mediate VDJ recombination.15,16 Consistently, the deletion of the negative PI3K regulator phosphatase and tensin homolog (PTEN) inhibits normal B cell development.15,21 However, constitutively active Foxo1 cannot rescue B cell development in PTEN-deficient mice suggesting that PI3K signaling also plays Foxo1-independent roles in B cell precursors.15 Recent studies demonstrate that Pten deletion compromises BCR-Abl1-or NRAS-driven leukemogenesis22 (Figure 2). Upon PTEN loss, B cell acute lymphoblastic leukemia (B-ALL) cells show increased glucose consumption and lactate production but decreased ATP levels.32 This need for PTEN expression in B-ALL cells is surprising considering that PTEN deletion in BCR-Abl1-driven chronic myeloid leukemia (CML) does not inhibit cell survival.32 Moreover, PTEN is known to play a role as a tumor suppressor in many other types of cancer including B cell lymphoma.33,34

In summary, these studies highlight the importance of metabolic checkpoint regulators in B cell precursors and demonstrate that perturbations in metabolic homeostasis are more detrimental for pre-B cells and their malignant counterparts than other cell types. In the model that emerges from these studies, developing B cells appear to anticipate the possibility of malignant transformation and display increased sensitivity toward metabolic stress to prevent malignant outgrowth. This sensitivity toward metabolic stress appears to be deeply rooted into B cell identity. Recent work from the group of Markus Müschen has shown that transcription factors that promote commitment to the B cell lineage also contribute to a constant state of energetic deprivation. PAX5 and IZKF have been shown to drive the expression of the glucose transport inhibitors NR3C1, TXNIP, and CNR2 and to induce the downregulation of glucose transporters GLUT1, GLUT3, and GLUT6.19 Not surprisingly, restriction of glucose uptake is disadvantageous for malignant B cells and genetic lesions inactivating PAX5 or IZKF1 are frequently found in B-ALL19 (Figure 2). Reconstitution of patient-derived B-ALL cells with wild-type PAX5 and IZKF has been shown to result in reduced glucose uptake, increased energetic stress, and decreased competitiveness in comparison with control cells.19 In addition to glucose uptake, B lymphoid transcription factors also determine into which metabolic pathway glucose is shunted. PAX5 has been shown to repress the expression of pentose phosphate pathway (PPP) enzymes such as glucose-6 phosphate dehydrogenase (G6PD) and to increase the expression of serine/threonine protein phosphatase 2A (PP2A).35 This expression profile fundamentally changes how B cells utilize glucose and how they maintain redox balance. The PPP, which branches from glycolysis, produces the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). NADPH plays an important role in ameliorating oxidative stress. Additionally, PPP is also needed for DNA replication by generating ribose 5-phosphate. Decreased G6PD expression thus reduces the flow of carbon through the PPP. To maintain adequate redox balance, B cells express PP2A, which redirects carbon from glycolysis toward the pentose phosphate pathway. As a consequence, PP2A limits glycolytic ATP production. The deletion of the PP2A subunit Ppp2r1a has been shown to increase glucose consumption, ATP levels, and lactate production in BCR-Abl1-transformed B-ALL but not CML. On the other hand, overall carbon flow through the PPP is reduced upon Ppp2r1a-deletion in B-ALL cells, the levels of NADPH/NADP are decreased, and production of reactive oxygen species (ROS) is increased demonstrating that PP2A plays an important role in the maintenance of redox balance. PP2A is needed for the survival of both normal and B-ALL cells demonstrating the importance of maintaining PPP activity through this pathway in B cells.35

In summary, these studies show that B cell precursors need to walk the fine line of supporting proliferation of normal cells and limiting oncogene-driven growth.

4 | METABOLIC QUIESCENCE IN MATURE B CELLS

Upon successful assembly of the BCR, immature B cells leave the bone marrow and migrate to the periphery to complete maturation in the spleen. Recent immigrants to the spleen are called transitional B cells and can be divided into several subsets based on their expression of surface markers and localization within the spleen.36 After maturation is complete, B cells either reside in B cell follicles of secondary lymphoid organs or circulate through the bloodstream. Mature B cells are largely inactive with few metabolic requirements, which is reflected by their small mitochondrial mass, low levels of glucose uptake, and relative insensitivity to inhibition of glucose metabolism.37 A recent study by Farmer et al38 suggests that acquiring a metabolically quiescent phenotype is essential for B cells to progress to the mature B cell stage in humans. This work demonstrates that in comparison with mature follicular B cells, transitional B cells show increased mitochondrial content, oxygen consumption, and ROS production. In addition to increased mitochondrial activity, transitional B cells are also characterized by higher levels of glucose uptake and lactate secretion, suggesting that transitional B cells are in general more metabolically active than mature B cells.38 Consistent with an increase in mitochondrial biogenesis and activity, transitional B cells show enhanced mTORC1-dependent signaling. Furthermore, the authors show that B cell maturation is accompanied by an increase in adenosine salvage metabolism and activation of AMPK. AMPK-mediated mTORC1 inhibition could be the underlying cause for the observed reduction in metabolic activity in mature human B cells.38

Considering this differences in metabolic activity between mature and transitional B cells, the question arises: What is the purpose of this metabolic silencing and what would the consequences be if developing B cells failed to enter metabolic quiescence? Since the re-arrangement of immunoglobulin genes in B cell precursors is random, a large fraction of newly developed B cells carry a B cell receptor recognizing autoantigens. Several checkpoints during B cell development and differentiation exist to eliminate or silence autoreactive B
cells. Although BCR signaling is essential for the development and maintenance of all B cells, a strong BCR signal can induce negative selection in transitional B cells. As a consequence, many B cells are lost at the transitional B cell stage and do not enter the mature B cell pool. Is aberrantly increased metabolic activation interpreted as an indication of autoantigen exposure in B cells and does it induce negative selection? In their work, Farmer et al describe patients with gain-of-function Pik3cd mutations displaying increased mTORC1 signaling. B cell development in these individuals is aborted at the transitional B cell stage (Figure 2). Mature B cells are nearly absent in the circulation of these patients suggesting that an inability to attenuate mTORC1 signaling impairs B cell development. Although murine B cells differ in some aspects of their development from human B cells, they do show a decrease in the expression of genes linked to protein synthesis and respiration when transitioning to the mature stage. Similar to human B cells, unrestricted mTORC1-activation leads to impaired B cell development and/or maintenance (Figure 2). Cd19cre × Tsc1flox mice display a relative accumulation of transitional B cells and a reduction of mature B cells. In these mice, the negative mTORC1-regulator tuberous sclerosis complex 1 (TSC1) is deleted beginning at the pro-/pre-B cell stage. However, the deletion of floxed alleles in the bone marrow in the Cd19cre × Tsc1flox background results in TSC1 deficiency in transitional B cells and leads to increased B cell survival. Thus, TRAF3 deficiency in B cells mimics chronic BAFF-R signaling and leads to increased B cell survival. Naive TRAF3-deficient B cells display increased Glut1 and hexokinase II expression as well as mitochondrial mass or ROS production is however not affected by the loss of TRAF3. In conclusion, excessive BAFF-R signaling achieved through increased BAFF availability or TRAF3 deletion results in increased metabolic activity, but instead of inhibiting B cell maturation boosts B cell survival. Considering that GSK3 inhibition and mTORC1 activation are downstream of BAFF-R-mediated signaling, it would be interesting to investigate why GSK3 and TSC1 deficiency interfere with B cell maturation or survival but chronic exposure to BAFF does not. Does BAFF-R activation or TRAF3 deficiency provide additional pro-survival signals that rescue metabolically stressed cells? Or do B cells from BAFF transgenic mice retain the ability to modulate GSK3 and mTORC1 activity and limit their effects on cell metabolism to a sustainable level? Answering these questions could help to identify metabolic vulnerabilities in autoreactive B cells to be exploited for treatment.

5 | METABOLIC BALANCE IN ACTIVATED AND GERMINAL CENTER B CELLS

When naive B cells encounter antigen, they need to rapidly rewire their metabolic program to foster the proliferative burst that follows activation. Many studies to this date have focused
on elucidating which intracellular signaling pathways support this metabolic reprogramming.\textsuperscript{7,8} Perhaps not surprisingly, many signaling pathways that are initiated during B cell activation simultaneously promote cell cycle progression, survival, and the acquisition of the appropriate metabolic profile. Consequently, the inability to activate these signaling pathways results in a failure to induce anabolic metabolism and thus prevents cell growth and proliferation. Questions that are less well investigated to this date however are as follows: How is metabolic homeostasis maintained in activated B cells? How do B cells return to a quiescent phenotype once they cease to proliferate? What happens if metabolic homeostasis is perturbed in activated B cells? How do activated B cells respond to low nutrient levels?

Thymus-dependent B cell immune responses take place in specialized lymphoid sites called the germinal centers (GC). Within the GC, B cells undergo somatic hypermutation of their immunoglobulin variable regions to increase antibody affinity.\textsuperscript{55} Since mutations introduced into the immunoglobulin genes are random, it is possible for GC B cells to lose specificity for the activating antigen. To prevent the expansion of unspecific or autoreactive clones, GC B cells are poised to undergo apoptosis and die quickly if they do not receive rescue signals. These signals include stimulation via CD40 and IL4 receptor and are delivered by the follicular helper T cells (Tfh).\textsuperscript{55} B cells interact with T cells, and other cell types such as the follicular dendritic cells, in the so-called “light zone” of the GC. The strength of the T cell-derived co-stimulatory signals is dependent on the ability of B cells to present antigen. B cells with BCRs binding with high affinity to antigen are more competent at presenting antigen to T cells and therefore receive more T cell help. Selected GC B cell clones subsequently enter the so-called “dark zone” of the GC to clonally expand. The final output of the GC reaction is the differentiation to antigen secreting plasma cells and memory B cells.\textsuperscript{55}

To prepare for proliferation in the dark zone, T cell-derived co-stimulatory signals induce the activation of metabolic regulators such as mTORC1 or c-Myc in GC B cells, which in turn promote cell mass acquisition.\textsuperscript{56,57} Myc expression in these cells is directly proportional to the amount of antigen presented and the strength of the Tfh signal.\textsuperscript{57} Interestingly, mTORC1 and c-Myc are activated in the light zone GC B cells and not in the highly proliferative population of dark zone B cells.\textsuperscript{56,58} Activation of these factors in the light zone seems to generate a metabolic reservoir that is subsequently used up in the dark zone. Once the resources are depleted, GC B cells stop proliferating and return to the light zone. The extent of T cell help thus regulates the number of cell cycles the GC B cells can undergo by defining the metabolic capacity of the cells prior to cell cycle entry.

In addition to c-Myc and mTORC1, GSK3 seems to play a role in regulating B cell metabolism and biomass synthesis in the GC. GSK3 is inhibited in a subset of GC B cells, which display c-Myc expression and show a signaling profile consistent with GC B cells undergoing positive selection.\textsuperscript{37} In resting cells, GSK3-deficient B cells are stimulated with anti-CD40 and IL4.\textsuperscript{37} This increase in metabolic activity translates into faster proliferation in vitro. Equally, if GSK3-deficient B cells are stimulated with anti-CD40 in vivo, their proliferation is increased in comparison with their wildtype counterparts. Surprisingly, however, GSK3-deficient cells are not able to participate in the GC response. If GSK3 is deleted during an ongoing GC response, the cells quickly die.\textsuperscript{37} This discrepancy in survival outcomes between CD40 stimulated B cells and GC B cells may arise from the fact that GSK3-deficient B cells are more susceptible to metabolic stress. In contrast to GC B cells undergoing selection in the light zone, dark zone GC B cells do not show Myc accumulation but instead express Foxo1.\textsuperscript{59,60} This transcription factor is needed to establish the dark zone gene expression profile.\textsuperscript{59,61} In other cell types, Foxo1 has been shown to drive a metabolically quiescent phenotype\textsuperscript{61}; however, it is not clear whether Foxo1 fulfills the same role in GC B cells.

In summary, although direct measurements of the metabolic pathways used by different GC B cells are currently lacking, it appears that GC B cells shift between phases of high anabolic cell growth and phases of reduced anabolic activity. Biomass acquisition seems to be directly dependent on co-stimulatory signals delivered by Tfh cells and to serve as an important factor in determining GC B cell capacity to divide in the dark zone.

The metabolic properties of GC B cells are however not only dependent on Tfh derived stimuli, but are also determined by the nutrient availability. While initial activation takes place in a nutrient rich environment, the following steps might be metabolically more challenging. GC B cells represent a highly proliferative population of cells, and it is possible that nutrients can become scarce during the course of the GC response. Although it is not clear whether key nutrients such as glucose or glutamine become limiting in the GC, it has been shown that B cells are exposed to hypoxia in the light zone of the GC.\textsuperscript{37,62,63} B cells stimulated under hypoxia show lower mTORC1 activation and reduced proliferation in comparison with B cells cultured under normoxia demonstrating that hypoxia affects B cell responses.\textsuperscript{62}

In conclusion, two types of events might reduce GC B cell anabolic metabolism: the absence of stimulating signals once the cells leave the light zone and a metabolically restrictive environment. What happens to B cells that fail to inhibit their metabolic activity? Would an escape from cytokine-regulated metabolism result in aberrant proliferation and malignant transformation? Or would these cells quickly outstrip available nutrients, experience metabolic stress, and succumb to apoptosis? Considering findings from recent years, these questions appear not to be straightforward to answer. As mentioned, GSK3 is an important metabolic regulator in the GC and its activity is dynamically regulated during the GC response. In comparison with control B cells, GSK3-deficient B cells show increased glucose uptake, cell mass accumulation, lactate secretion, mitochondrial mass acquisition, and oxygen consumption if stimulated with anti-CD40 and IL4.\textsuperscript{37} This increase in metabolic activity translates into faster proliferation in vitro. Equally, if GSK3-deficient B cells are stimulated with anti-CD40 in vivo, their proliferation is increased in comparison with their wildtype counterparts. Surprisingly, however, GSK3-deficient cells are not able to participate in the GC response. If GSK3 is deleted during an ongoing GC response, the cells quickly die.\textsuperscript{37} This discrepancy in survival outcomes between CD40 stimulated B cells and GC B cells may arise from the fact that GSK3-deficient B cells are more susceptible to metabolic stress.
low glucose medium, GSK3-deficient B cells show decreased viability in comparison with control B cells.37 By extension, perhaps in the metabolically stressful environment of the GC, unrestrained metabolic activity becomes fatal for GSK3-deficient B cells. Interestingly however, c-Myc transgenic B cells are able to form GCs.37 Similarly to GSK3-deficient B cells, c-Myc transgenic B cells show increased proliferation, glucose consumption, and cell mass acquisition in vitro, yet do not replicate the phenotype of GSK3-deficient B cells in vivo.37 Since c-Myc is stabilized in GSK3-deficient B cells, other signaling pathways activated downstream of GSK3 inhibition must be responsible for the observed reduction in cell viability. Akin to c-Myc transgenic B cells, B cells with increased PI3K signaling are able to participate in the GC response. Different mouse models exist in which PI3K signaling is enhanced through the deletion of negative regulators of this signaling pathway. In one of these models, tandem PH domain–containing proteins (TAPP) are mutated so they cannot bind their phosphoinositide ligand PI(3,4,5)P2. Stimulated B cells from these mice show markedly increased activation of the PI3K regulated kinase Akt.64 Moreover, these cells show increased GSK3 inhibition and phosphorylation of the mTORC1 target molecule 70S6K. B cells with mutant TAPP molecules show an increased expression of the glucose transporter Glut1 and enhanced glucose uptake in a PI3K-dependent manner. Overall, these cells exhibit a hypermetabolic phenotype after stimulation, consume increased levels of oxygen, secrete more lactate, and accumulate more biomass than their wild-type counterparts.64 This hypermetabolic phenotype however does not result in an aborted GC response. Instead, TAPP knockin mice develop chronic GCs and symptoms of autoimmunity.64 GC B cell viability is also not affected in other mouse models with B cells exhibiting increased PI3K signaling, such as the PTEN-deficient mice.65

An important metabolic regulator activated downstream of PI3K signaling is mTORC1. As mentioned before, mTORC1 activity is temporally induced in GC B cells undergoing T cell–dependent selection. Conflicting results have been published on the phenotype of TSC1-deficient B cells; however, two out of three studies report unrestrained mTORC1 activity to reduce the competitiveness of GC B cells52,63,66 (Figure 2). The TSC complex, which consists of TSC1 and TSC2, regulates mTORC1 activity primarily in response to mitogenic stimuli. Akt has been shown to inhibit the TSC complex, and GSK3-mediated TSC phosphorylation leads to its activation.46,66 (Figure 1). Thus, in activated B cells different signaling pathways converge on TSC inhibition and mTORC1 activation. In normal B cells, mTORC1 activity is not only dependent on growth factor–mediated signaling, but also controlled by nutrient availability and energy levels. Nutrient sensing by mTORC1 involves the heterodimeric complex of small GTPases RagA and RagC (Figure 1). RagA and RagC together with other molecules form a supercomplex at the surface of lysosomes that recruits and activates mTORC1 under nutrient sufficiency.67 Constitutively active RagA renders mTORC1 signaling independent of the presence of amino acids and has been shown to reduce B cell competitiveness in the GC56 (Figure 2). In contrast, expression of mutant alleles of the gene RagC that drive partial but not complete uncoupling of mTORC1 activity from amino acid dependence has been shown to boost GC expansion.68 (Figure 2).

Nutrient and energy levels are also sensed by the LKB1/AMPK pathway, which impinges on mTORC1 activity. The role of the LKB1/AMPK signaling pathway in GC B cells is complex and extends beyond the role of metabolic regulation. LKB1 has been shown to be activated downstream of DNA double-strand breaks and to be needed for plasma cell differentiation.22 Additionally, LKB1 inhibits NFκB signaling and IL6 secretion.22 Due to altered cytokine secretion, B cell–specific Lkb1 deletion results in T cell hyperactivation and spontaneous GC formation. Interestingly however, the generated GCs are mostly cells that have escaped Lkb1 deletion suggesting that Lkb1-sufficient cells outcompete Lkb1-deficient cells in the GC.22 While it is not clear whether LKB1 is also important for GC B cells to respond to metabolic stress, these findings demonstrate that the ability to restrict cell growth in response to stress signaling in general is essential for an appropriate GC response. Moreover, since LKB1 drives plasma cell differentiation, it would be interesting to analyze whether the induction of differentiation is a general mechanism for B cells to escape metabolic stress. Among other targets, LKB1 regulates B cell metabolism by activating AMPK. However, AMPKα1 ablation does not affect B cell proliferation, nutrient uptake, or GC formation suggesting that other LKB1 targets play a more prominent role in GC B cells.21

Lastly, a typical adaptive response to low oxygen levels is the stabilization of the transcription factor hypoxia-inducible factor 1α (Hif1α) and a metabolic shift toward glycolysis.69 Consistent with the GC being a hypoxic environment, GC B cells have been found to accumulate Hif1α.37,62,63 Interestingly, Hif1α hyperstabilization has been shown to be detrimental to GC B cell survival.65 B cells deficient for the von Hippel-Landau tumor suppressor protein (pVHL) yield fewer GCs during an immune response and produce lower levels of high affinity antibodies.62 Under sufficient oxygen conditions, pVHL destabilizes Hif; pVHL deletion thus leads to a persistent hypoxic-signaling profile. These findings suggest that hypoxia serves as a break on the GC B cell response. Many questions remain however unanswered. Does initial Hif1α activation benefit GC B cells and enable them to generate energy in an oxygen-depleted milieu? How are T cell–derived signals integrated into this model? Are GC B cells actively receiving T cell help better equipped to resist hypoxia-induced mTORC1 inhibition?

In summary, while the dynamic expression and activation of metabolic regulators in the GC suggest that anabolic cell growth is tightly regulated in this subset, a comprehensive model of how B cells maintain metabolic homeostasis is still missing. Dysregulation of specific metabolic regulators can have vastly different effects on GC B cells survival and proliferation despite the fact that these signaling molecules are intimately connected in their function and often drive a similar hypermetabolic profile.

### 6 | MALIGNANT B CELLS AND CELLULAR SENESCENCE

Similarly to pre-B cells, GC B cells are at a particularly high risk of undergoing malignant transformation. GC B cells manifest phenotypic
features such as massive proliferation, genome instability, and resistance to DNA damage that create an inherent risk for malignant outgrowth.\textsuperscript{3} A testament to the special role GC B cells play in lymphomagenesis is the fact that the majority of B cell neoplasms are derived from GC-experienced B cells.\textsuperscript{5}

Cancer cells are characterized by increased metabolic activity and rapid proliferation. Unlike normal B cells, malignant B cells proliferate even in the absence of extracellular growth signals due to aberrant activation of intracellular pro-survival or pro-proliferation signaling pathways. Dysregulated expression or activation of a single oncogene is however usually not sufficient to result in malignant transformation. One mechanism through which aberrant proliferation can be aborted is oncogene-induced senescence (OIS). Senescence is a cellular process characterized by a stable cell cycle arrest that plays a role in different physiological processes, contributes to aging, and serves as a failsafe mechanism to prevent tumorigenesis. A number of molecular effector mechanisms of senescence have been identified to date, suggesting that senescence is a cellular phenotype that can be caused and executed by different molecular events.\textsuperscript{70} Among other factors, metabolic imbalance has been shown to contribute to OIS.\textsuperscript{71,72} Oncogene-driven cell growth substantially increases the need for energy and biosynthetic precursor molecules thereby creating metabolic stress. Only cells that are capable of bypassing this stress can evade growth arrest and progress toward malignant transformation. Infection of B cells with the Epstein-Barr virus (EBV) has been shown to result in an initial wave of proliferation that is aborted shortly afterward, and the majority of infected cells exit cell cycle. Growth arrested cells have been shown to increase the expression of sestrins leading to AMPK activation and mTORC1 inhibition.\textsuperscript{73} Activation of these metabolic stress-signaling pathways suggests that the cells failed to satisfy their high metabolic demands. Providing the cells with cell-permeable metabolites has been shown to increase the number of proliferating cells, demonstrating that metabolic stress contributes to limiting EBV-mediated replicative capacity.\textsuperscript{73} How exactly metabolic stress responses are coupled to senescence induction in B cells remains poorly defined. Senescent cells in general do not show a metabolic phenotype similar to quiescent cells, but are often enlarged, increase consumption of oxygen and produce more ROS. Persistent mTORC1 activation and cell mass accumulation in cell cycle–arrested cells have been suggested to lead to senescence induction\textsuperscript{74} and to contribute to the functional decline in senescent cells.\textsuperscript{75} AMPK has also been suggested to play a role in the induction of senescence with temporarily restricted AMPK induction allowing adaptation to stress and persistent AMPK activation leading the cells toward irreversible senescence.\textsuperscript{75} This prompts the question: What is the role of these metabolic checkpoints in B lymphoma development and propagation? What are the molecular mechanisms allowing B cells to evade senescence? How do premalignant and malignant B cells navigate the decision between cell death, quiescence, and senescence when exposed to metabolic stress? Considering our current knowledge on the role of metabolic checkpoint regulators in B cells, these questions cannot be unequivocally answered at this time.

As discussed above, AMPKα1 deletion in Myc-driven lymphoma accelerates lymphoma development by boosting mTORC1 activity, biosynthesis, and Hif1α-dependent glycolytic activity.\textsuperscript{28} Interestingly, while Hif1α is not essential for the survival of normal lymphoma cells, AMPKα1-deficient cells become addicted to this transcription factor.\textsuperscript{28} The deletion of the AMPK-interacting protein Fnip1 in the same mouse model prevents lymphoma genesis. Fnip1-deficient B cells have been shown to retain high mTORC1 signaling even when AMPK activity is induced.\textsuperscript{20} Similar to AMPK, the loss of LKB1 has been suggested to contribute to lymphoma development. Mice with a B cell–specific LKB1 deletion (Cd19cre × Lkb1flox mice) spontaneously develop tumors despite impaired survival of mature B cells\textsuperscript{77} (Figure 2). In addition to AMPK, RagC/RagA GTPases control mTORC1 activity in response to nutrient deprivation. While RagA mutations are rare, RagC mutations leading to reduced dependence of mTORC1 signaling on amino acids are frequently found in follicular lymphoma cells.\textsuperscript{68} Expression of these mutants in a mouse model in which the pro-survival factor Bcl2 is overexpressed accelerates lymphoma development\textsuperscript{68} (Figure 2). These findings suggest that partial dysregulation of AMPK/mTORC1 activity benefits lymphoma cells, but should not exceed a certain threshold. The role of LBK1/AMPK as tumor suppressors in lymphoma cells is in stark contrast to the previously discussed dependence of leukemic cells on these factors. In order to better understand the role of AMPK/LBK1 in B cell–derived malignancies, it would be important to elucidate how the maturation status or the transforming oncogene influences how metabolic homeostasis is maintained in B cells.

A similarly complex picture emerges when studying the role of GSK3 in B cell lymphoma. As discussed above, GSK3 is a metabolic regulator, needed to maintain cellular quiescence. Upon GSK3 inhibition, numerous pro-survival and pro-proliferation factors are stabilized. In some cell types, GSK3 can act as a tumor suppressor; however, GSK3 has also been implicated to have tumor promoter effects in certain types of cancer.\textsuperscript{76} Although GSK3 deletion in mature B cells leads to increased proliferation, these cells are more sensitive to metabolic stress and fail to participate in the GC response.\textsuperscript{37} In contrast to GC B cells, a recent study suggests that Myc-driven lymphoma benefits from GSK3 inhibition.\textsuperscript{77} Varano et al demonstrate that lymphoma cells lacking surface BCR display an altered metabolic profile and are less competitive than BCR-sufficient cells. The authors suggest that BCR-derived signaling attenuates GSK3 activity thereby supporting a beneficial metabolic profile. Using a pharmacological GSK3 inhibitor, the authors show that GSK3 inhibition can rescue competitiveness of BCR-deficient lymphoma cells.\textsuperscript{79} These findings suggest that GSK3 plays a tumor suppressor role in B cell lymphoma. On the other hand, it has been shown that the GSK3 inhibitor 9-ING-41 induces cell death in numerous lymphoma lines in vitro and in vivo.\textsuperscript{80} Moreover, increased GSK3 expression in B cell lymphoma patients correlates with reduced overall survival arguing for a tumor promoter role of GSK3.\textsuperscript{80} Since GSK3
has many different target molecules and affects a plethora of biological processes, it is possible that the signaling context of the given cell determines the outcome of GSK3 inhibition. A model that would help predict under which conditions GSK3 inhibition induces cell death rather than boosting metabolic activity is crucial in order to be able to use GSK3 inhibitors to treat lymphoma patients.

In summary, the discussed studies emphasize the complexity of metabolic homeostasis regulation in B cells. In order to successfully expand, transformed B cells need to bypass different checkpoints. They need to evade senescence induction while maintaining high levels of metabolic activity without completely depleting their energy stores. The signaling context of the given malignancy and the physiological niches these tumors occupy in vivo could determine how the cells respond to metabolic stress. A better understanding of the specific roles different metabolic regulators play in lymphoma biology would be rewarding in improving cancer treatment and disease stratification.

7 | METABOLIC HOMEOSTASIS IN PLASMA CELLS AND MEMORY B CELLS

Plasma cells are terminally differentiated effector cells that secrete large amounts of antibodies to neutralize pathogens. Although these cells do not proliferate, their metabolic profile differs dramatically from naive B cells. Since plasma cells can secrete thousands of antibody molecules per second, they require enormous quantities of different biosynthetic precursor molecules and additional carbon sources to generate energy.9 The metabolic program of plasma cells varies depending on the niches they occupy and depending on their longevity. Both short- and long-lived plasma cells have been shown to take up glucose and to use it for protein glycosylation.81 Long-lived plasma cells are able to use glucose-derived pyruvate to fuel the TCA cycle, and this metabolic feature plays an important role in promoting their survival.81 Although it is currently unclear how these different metabolic programs are established in plasma cells, their dependence on glucose metabolism highlights potential targets amenable to medical intervention. In addition to glucose, plasma cells also consume amino acids to enable biomass synthesis. The activity of the transcriptional repressor protein, Blimp1, which is important for the establishment of the plasma cell transcription program, favors the expression of amino acid transporters, and at the same time represses the transcription of AMPK activators sestrins 1 and 3.82 Although some insight exists on which signaling pathways are needed to supply plasma cells with nutrients, much less is known about how plasma cells maintain metabolic homeostasis and how they cope with metabolic dysregulation. One process involved in regulating metabolic homeostasis that has been shown to play an important role in plasma cells is autophagy. During autophagy, misfolded proteins and defective organelles are engulfed in specialized vesicles called autophagosomes, which fuse with lysosomes leading to the degradation of the autophagosome contents. This process requires the coordinated action of a set of specialized proteins to recruit cargo, to assemble the autophagosome, and to induce the fusion of autophagosomes and lysosomes.83 Originally, autophagy has been viewed as a means to gain energy during periods of starvation. It is becoming increasingly apparent however that autophagy is also needed in order to remove damaged organelles and toxic protein aggregates. Plasma cells face increased proteotoxic and oxidative stress due to their high levels of protein synthesis. The lack of an essential autophagy molecule, Atg5, results in an expansion of the endoplasmic reticulum (ER) and increased antibody secretion in plasma cells.84 However, these cells show reduced energy levels and a shortened life span. Thus, autophagy represents a trade-off between antibody generation and viability in plasma cells.84 In a similar manner, autophagy has been found to be essential for the survival of B1a cells. B1a cells are a unique long-lived B cell subset providing first-line defenses against many common pathogens by secreting the so-called “natural antibodies.” The deletion of Atg7 in B cells has been found to result in the loss of B1a cells, without affecting conventional B cells.10

Autophagy has also been shown to play a crucial role in the survival of memory B cells. Memory B cells represent a long-lived population of antigen-experienced cells, which are able to quickly respond to a secondary antigen challenge. Mice with a B cell-specific deletion of Atg7 show normal primary antibody responses, but fail to generate protective memory responses.85 The longevity of memory B cells seems to be dependent on efficient removal of damaged cellular components since Atg7 deficiency results in cell death in this population.

In summary, autophagy plays a crucial role in B cell subsets with high rates of protein secretion or in long-lived cells. Autophagy is closely linked to the signaling circuitry coordinating nutrient availability and cell growth and inhibited by mTORC1 if nutrients are abundant86 (Figure 1). How the various autophagy-dependent B cell subsets respond to mTORC1 dysregulation is however poorly understood to this date.

8 | CONSEQUENCES OF DISRUPTED METABOLIC HOMEOSTASIS

Metabolic stress occurs when the cells' nutrient uptake or energy generation does not match the cells' biosynthetic needs. This is the case if the rate of cell growth exceeds the cells' capability to take up or process nutrients, or if insufficient nutrients are present in the environment. Cells can respond to this stress by either increasing the expression of metabolic transporters and rate-limiting metabolic enzymes or by inhibiting energy-consuming processes such as cell growth. As discussed above, in certain situations B cells are not able to adapt to metabolic stress, which then can become detrimental. In order to manipulate B cell immune responses in the context of human diseases, it is important to understand the mechanisms that lead to B cell death if metabolic homeostasis is disrupted. An inability to inhibit energy-consuming processes or a failure to increase energy production may simply result in an energetic collapse. Insufficient ATP supply will halt many vital cellular
functions and cells will ultimately succumb to apoptosis. In many instances however, the absence of metabolic regulators does not result in an acute depletion of energy stores, but rather impacts on viability over a longer period of time. Increasing evidence suggests that ROS production provides a link between cell metabolism and cellular life span. A significant portion of intracellular ROS is produced as a consequence of normal mitochondrial function (Figure 3). The electron transport chain (ETC) transfers...
electrons from NADH or FADH2 in a series of steps to oxygen, the ultimate electron acceptor. The energy gained from this transfer is used to pump protons across the inner mitochondrial membrane, to build up an electrochemical gradient. This gradient is subsequently used to drive ATP synthase activity to generate ATP. This process is not error free and electrons can prematurely exit the ETC. Electron leakage primarily occurs at complex I and complex III of the ETC resulting in a partial reduction of oxygen to superoxide. Mitochondrial dismutase subsequently converts superoxide to hydrogen peroxide, which is free to diffuse into the cytosol. Consistent with ROS production being associated with normal mitochondrial function, enhanced oxygen consumption often correlates with increased ROS production in B cells. Various cellular stresses that affect mitochondrial health, the rate of electron flux through the ETC, the concentration of electron carriers, mitochondrial membrane potential, oxygen availability, and the type of substrate being oxidized can also augment ROS production.

A second source of ROS that may play an important role in B cell biology is protein folding in the ER (Figure 3). Formation of immunoglobulins, which are rich in disulfide bonds, can lead to significant ROS production. In professional secretory cells such as plasma cells, the ER can represent a major source of intracellular ROS. Additional sources of ROS include plasma membrane-associated NADPH oxidases (NOX) or dual oxidases (DUOX) (Figure 3). Crosstalk between these different ROS producing sites can result in a feed-forward loop amplifying an originally localized ROS signal.

Under physiologic conditions, ROS play an important role in BCR-mediated signaling and B cell fate decisions. ROS are produced in response to BCR stimulation and can reversibly oxidize different signaling molecules such as protein tyrosine phosphatases SHP-2 and PTEN. Oxidation of these proteins leads to reduced phosphatase activity and thus enhanced signal transduction. ROS is absolutely crucial for BCR-mediated signaling as ROS scavengers prevent BCR-ligation induced proliferation. Additionally, although not without controversy, ROS has been suggested to affect B cell differentiation to plasma cells.

While playing an indispensable role in propagating BCR signaling, excessive ROS has been shown to have the opposite effect and to inhibit BCR responses. Moreover, ROS can reduce cell viability by inducing DNA damage and lipid peroxidation. Excessive ROS therefore interferes with normal B cell function and needs to be neutralized to avoid oxidative injury. To this end, several enzymatic and non-enzymatic systems exist in B cells that correct redox imbalance (Figure 3). The two major antioxidant pathways in B cells are the glutathione and thioredoxin (Trx) systems. Glutathione exists in two forms: reduced glutathione (GSH) and oxidized glutathione (GSSG). GSH can remove peroxide, superoxide, and other radicals and is transformed into GSSG that can be recycled to GSH by the action of glutathione reductase. Reduced thioredoxins are potent reductases with a relatively broad spectrum of substrates that partially overlaps with the glutathione system. The thioredoxin and glutathione systems have been shown to play crucial but partially redundant roles in B cells. NADPH produced by the PPP acts as an electron donor for both the thioredoxin and glutathione pathway.

Thus, PPP activity plays an important role in the maintenance of redox homeostasis. The synthesis of glutathione is dependent on the availability of the amino acid cysteine. In plasma, cysteine is present mainly in its oxidized form cystine and transported into cells via the amino acid antiporter system x_c^-. B cells have been shown to express low levels of x_c^- components and are thus dependent on receiving cysteine from other cells. The cysteine/cystine redox cycle does not only regulate GSH levels, but can also protect cells from lipid peroxidation and can be viewed as a regulator of redox balance by itself.

Pentose phosphate pathway activity, cysteine import, GSH, and thioredoxin production represent processes that can be fine-tuned to match the cells’ current needs. Additionally, B cells can mitigate ROS-induced damage through autophagy or through other mechanisms such as mitochondrial fusion and fission. Thus, B cells possess different means to maintain redox balance. Normal B cells with a high risk of oxidative stress are known to upregulate their antioxidant defense machinery. How B cells respond to oxidative stress stemming from metabolic dysregulation is however less well understood. There is precedent for ROS-induced cell death in B cells with dysregulated metabolic homeostasis. Activated GSK3-deficient B cells cultured under glucose low conditions have been shown to accumulate ROS and to display reduced viability in comparison with wildtype cells. The survival defect can be however rescued by providing the cells with the ROS scavenger N-acetyl cysteine. In a similar manner, HuR-deficient B cells show ROS accumulation and reduced survival and proliferation that can be partially rescued by the addition of ROS scavengers to the culture. HuR is a RNA-binding protein that affects the splicing of a vast array of mRNA transcripts and is essential for GC B cell responses. The exact mechanism of ROS accumulation in the absence of HuR is not known yet, but may include reduced TCA cycle activity due to impaired splicing of mRNA that encodes dihydrolipoamide S-succinyltransferase.

Autophagy deficiency has also been reported to result in ROS accumulation with fatal consequences for memory B cells. Atg7-deficient memory B cells show reduced mitochondrial membrane potential, increased ROS production, and reduced viability in comparison with wildtype cells. The viability of Atg7-deficient memory B cells can be rescued by providing the cells with antioxidants suggesting that autophagy plays a vital role in the clearance of dysfunctional mitochondria.

In summary, compelling evidence exists that ROS production as a result of metabolic dysregulation can significantly contribute to the disruption of B cell survival and function. Analysis of B cell metabolism should therefore include the assessment of the activity of ROS scavenging pathways. The balance between ROS production and the antioxidant system could be the tipping point between adaptation to metabolic stress and cell death and should therefore be taken into consideration when the role of B cell metabolism in cell fate and function is evaluated.

**9 | CONCLUSIONS**

Our understanding of how B cells respond to metabolic hyperactivation is currently incomplete. Metabolic activity in B cells is governed...
by two factors: growth signals and nutrient availability. An escape from growth factor-dependent regulation of anabolic metabolism can lead to different outcomes: The cells can undergo cell death, can become senescent, or can be pushed toward malignant transformation. We have yet to establish a model that would reconcile the different observations made on the effects of aberrant activity of the major metabolic regulators c-Myc, mTORC1, GSK3, and AMPK on B cell viability and function. One could envision that different factors contribute to how B cells respond to a mismatch between metabolic demand and the availability of resources. The signaling context and differentiation status could be one component that determines the outcome of metabolic hyperactivation. The ability to activate anti-oxidant defense mechanisms or to increase the expression of nutrient transporters and key elements of the metabolic machinery could play an important role in this regard. Additionally, activation of pro-survival rescue signals might be needed to allow B cells with increased metabolic activity to persist. Moreover, the duration and strength of the stress signal could contribute to the functional outcome of c-Myc or mTORC1 hyperactivation. A layer of complexity is added by the fact that all of the discussed molecules have other functions beyond metabolic control. Untangling their different roles in cell cycle progression, B cell development, and effector function is challenging and will require further investigation.

A better understanding of how B cells respond to metabolic stress is crucial in order to develop new treatment strategies for B cell–derived cancer and for autoimmune disorders. Both malignant and autoreactive B cells are characterized by aberrant metabolism supporting their proliferation and survival. Identifying metabolic weaknesses of these cells could open new opportunities for therapeutic intervention. In a similar manner, compounds altering the activity of various metabolic regulators are available and could be used for treatment. As an example, GSK3 inhibitors are used to treat various disorders and have been considered for the treatment of myeloid leukemia.\textsuperscript{104,105} Further insight into the signaling pathways, which render B cells susceptible to GSK3 inhibition, could provide a rationale to extend the use of GSK3 inhibitors to B cell–derived malignancies or improve disease stratification. Lastly, since it is becoming increasingly apparent that B cell metabolism and function are not only regulated by intracellular signaling but also by environmental cues, it is important to assess how B cell responses are altered in response to changes in whole-body metabolism. Would GSK3 inhibition have a different effect in patients with diabetes or obesity? Is there a benefit in restricting food intake for particular B cell–derived disorders? Many questions centering on B cell metabolism await future investigation. The last few years have brought an increased appreciation of the role metabolism plays in guiding B cell fate and function. The challenge for the future lies in developing a comprehensive model of adaptive stress responses that would allow us to predict how manipulation of specific signaling pathways affects B cell survival in a changing metabolic environment.

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CONFLICT OF INTEREST

None.

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