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B cell identity as a metabolic barrier against malignant transformation

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Abstract

B-lineage and myeloid leukemia cells are often transformed by the same oncogenes, but have different biological and clinical characteristics. While B-lineage acute lymphoblastic leukemia (ALL) cells are characterized by a state of chronic energy deficit, myeloid leukemia cells show abundant energy reserve. Interestingly, fasting has been demonstrated to selectively inhibit the development of B-lineage ALL, but not myeloid leukemia, further suggesting that lineage identity may be linked to divergent metabolic states in hematopoietic malignancies. B-lymphoid transcription factors IKZF1, EBF1 and PAX5 are essential for early B cell development and commitment to B cell identity. However, in >80% of human pre-B ALL cases, the leukemic clones harbor genetic lesions of these transcription factors. The significance of these defects has only recently been investigated. Here we discuss the unexpected function of a B-lymphoid transcriptional program as a metabolic barrier against malignant transformation of B cell precursor cells. The metabolic gatekeeper function of B-lymphoid transcription factors may force silent pre-leukemic clones carrying potentially oncogenic lesions to remain in a latent state. In addition, this program sets the threshold for responses to glucocorticoids in pre-B ALL. Finally, the link between tumor suppressor and metabolic functions of B-lymphoid transcription factors is matched by observations in clinical trials: Obesity and hyperglycemia are associated with poor clinical outcome in patients with pre-B ALL.

A mouse model to demonstrate potential link between obesity, LEPR and B-lineage ALL

Several lines of evidence suggest that glucose and energy supply represents a rate-limiting factor in oncogenic transformation of B cells. For instance, obese children and children with type 2 diabetes mellitus (T2DM) with higher glucose and insulin serum levels are more likely to relapse with B cell lineage acute lymphoblastic leukemia (ALL). They have

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significantly inferior outcomes compared to children with normal glucose/insulin levels [1–3]. In addition, pre-B ALL patients with high glucose levels at the time of induction chemotherapy were less likely to achieve durable remissions of disease, and had worse overall outcomes compared to those with normal glucose levels [4]. Likewise, previous studies demonstrated that obesity was associated with increased risk for patients with mature B cell lymphoma, including non-Hodgkin lymphoma (B-NHL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL) and also in multiple myeloma [5–7]. Obesity is associated with leptin resistance characterized by attenuated leptin receptor (LEPR) signaling [8]. Interestingly, positive associations were reported between incidence of B-NHL and polymorphisms in genes encoding molecules that regulate energy homeostasis, including leptin, LEPR and adiponectin [6,9]. Collectively, these findings suggest that high abundance of glucose and energy supply in the context of obesity and T2DM represents a risk factor for patients with B cell malignancies.

Studying a mouse model of *N-Myc*-driven B-cell lineage ALL, a link between obesity, Lepr signaling and development of ALL has recently been identified [10]. Notably, fasting selectively inhibited the development of B-lineage ALL, but not myeloid leukemia, through induction of Lepr expression. Furthermore, fasting arrested growth of B-lineage ALL in a human xenograft model, and expression levels of *LEPR* were found to be lower in human ALL samples in comparison to normal bone marrow samples [10]. Thus, downregulation of LEPR signaling contributes to development and maintenance of ALL, and the impacts of fasting are cell-type dependent, raising the question whether lineage identities (myeloid vs. B-lineage) are coupled with distinct metabolic states and control in hematopoietic malignancies.

Divergent clinical characteristics of B-lineage and myeloid malignancies

B-lineage and myeloid malignancies arise from lesions affecting oncogenes (e.g. *BCR-ABL1*, *RAS*, *MYC*, *MLL*) in multipotent progenitor cells. While often transformed by the same oncogenes, B-lineage and myeloid leukemias are markedly different in clinical characteristics [11–13]. For instance, while > 95% of chronic myeloid leukemia (CML) patients achieve long-term disease-free survival under treatment with tyrosine kinase inhibitors [12], patients with Philadelphia chromosome-positive (Ph^+) ALL invariably relapse within months after initial remission [13]. In addition, glucocorticoids (GCs) are highly active in the treatment of pre-B ALL and B cell lymphoma [14], while patients with myeloid leukemias do not benefit from GC treatment [15]. Furthermore, a recent study demonstrated that GCs favor survival of hematopoietic stem and myeloid progenitor cells [16], which is in striking contrast to induction of cell death in B cells [17].

Divergent responses to GCs in B-cell vs. myeloid malignancies represent an empirically established standard in clinical medicine; however, the underlying reason for this difference has only recently been investigated. Expression levels of the glucocorticoid receptor (NR3C1) depend on a B-lymphoid transcriptional program. The B-lymphoid transcription factors PAX5 and IKZF1 set the threshold for GC responses in patient-derived pre-B ALL cells [18]. It was previously reported that phosphorylation of NR3C1 by AKT1 inhibits nuclear translocation of the glucocorticoid receptor upon GC treatment, and that

pharmacological inhibition of AKT1 reverses GC resistance in ALL [19]. In addition to positively regulating *NR3C1* expression, inducible reconstitution of PAX5 or IKZF1 in patient-derived haploinsufficient pre-B ALL cells reduced phosphorylation of AKT-S^{473} [18], suggesting that PAX5 and IKZF1 may also modulate sensitivity to GCs through suppression of AKT activity.

B cell precursors are defined by a state of chronic energy depletion

With respect to their biological characteristics, B-lineage and myeloid leukemia cells differ in their proliferation kinetics, cell size, cytoplasmic volume as well as mitochondrial number and volume (TABLE 1). Compared to myeloid leukemia, pre-B ALL cells divide faster (5- to 24-fold shorter doubling-times), are significantly smaller (5-fold smaller cytoplasmic volume) and carry half the number of mitochondria with about one-third of the mitochondrial volume observed in myeloid leukemia cells (TABLE 1). Similarly, patient-derived myeloid leukemia cells have abundant ATP reserves (low AMP:ATP ratios), while AMP:ATP ratios in patient-derived pre-B ALL cells are very high and inverted compared to myeloid leukemia cells [18; Table 1]. High baseline AMP:ATP ratios indicate a state of chronic energy deficit which triggers constitutive activation of the energy-stress sensor LKB1-AMPK pathway [20]. Low baseline levels of ATP as well as restriction of mitochondrial volume and number suggest that B cell precursors are defined by a state of chronic energy depletion [18].

Genetic lesions in B-lymphoid transcription factors represent near-obligate defects in human ALL

Myeloid (*CEBPA, PU.1, GATA2*) and B-lymphoid (e.g. *IKZF1, EBF1, PAX5*) transcription factors determine myeloid vs. B-lineage identity, respectively. B-lymphoid transcription factors antagonize myeloid differentiation as an alternative lineage fate by repressing the myeloid master regulator CEBPα and *vice versa* [21–25]. The transcription factors *IKZF1*, *EBF1* and *PAX5* are critical for B cell development at the level of B-lineage commitment, V(D)J recombination and pre-B cell receptor signaling [26]. IKZF1 primes lymphoid gene expression in multipotent progenitor (MPP) cells, allowing differentiation into common lymphoid progenitors [CLPs; 27], and it is also required for pre-BCR signaling [28]. EBF1 is essential for the formation of pro-B cells and restricts lineage fate option. EBF1 antagonizes expression of myeloid transcription factors *CEBPα* and *PU.1*, while positively regulating expression of *PAX5* [24]. PAX5 is essential for the commitment to the B cell lineage and maintenance of B-cell identity [21].

While myeloid leukemia cells frequently acquire genetic lesions in myeloid transcription factors [29], pre-B ALL clones often carry genetic lesions that results in reduced activity or inactivation of B-lymphoid transcription factors [18,30,31]. Such genetic lesions may reduce the stringency of B-cell or myeloid lineage commitment; however, the significance of genetic inactivation of B-lymphoid transcription factors has only been recently elucidated. Studying patient samples from clinical trials for pre-B ALL in children and adults, inactivating lesions in B-lymphoid transcription factors *PAX5*, *IKZF1*, *EBF1* and *TCF3* were identified in the vast majority of ALL cases studied [18]. These lesions resulted in

decreased protein levels of PAX5 as well as expression of truncated dominant-negative IKZF1 proteins (IK6) owing to intragenic deletions of *IKZF1* [18]. Therefore, defects in B-lymphoid transcription factors represent near-obligate lesions in human pre-B ALL. Interestingly, CEBPA not only promotes myeloid development at the expense of B-lymphoid differentiation, but also functions as a mediator of glucose uptake in adipocytes [32]. Conversely, *Ebf1*$^{-/-}$ mice exhibit increased glucose transport and glucose transporter type 4 (GLUT4) expression in adipose tissues [33], suggesting that EBF1 may function as a negative regulator of glucose transport.

PI3K-AKT signaling couples B cell fate with metabolic state through regulation of transcription factors FOXO1 and IRF4

Previous studies demonstrated that hematopoietic stem and progenitor cells differ in their dependency on glycolysis [34]. More recently, asymmetric transmission of nutrient-sensing PI3K/AKT/mTOR signaling has been shown to dictate B cell fate through regulation of transcription factors FOXO1and IRF4 [35]. The transcription factor FOXO1 is known to induce *PAX5* expression and promote B cell lineage commitment [36]. AKT-mediated phosphorylation causes export of FOXO1 from the nucleus to the cytoplasm, inhibiting the transcriptional activity of FOXO1 [37]. While expression of *PAX5* is downregulated upon B cell differentiation into plasma cells [38], IRF4 mediates plasma cell differentiation [39]. In asymmetric cell divisions of B cells, high PI3K-AKT signaling characterized by FOXO1 inactivation, IRF4 induction and repression of PAX5 drives plasma cell differentiation. In contrast, the sibling cells with lower PI3K-AKT signaling undergo self-renewal and exhibit features of a memory or germinal center B cell fate [35].

Both IRF4 and FOXO1 were previously implicated in regulation of glucose and energy metabolism. For instance, IRF4 was shown to activate expression of glycolytic genes, including *Glut1/3*, *Hk2*, *Pfkfb3*, *Pfkm*, *Pfkp*, *Pgk1*, *Pgm2*, *Pgm2l1*, *Eno1* and *Ldh1*, in activated B cells [35]. On the contrary, FOXO1 plays a key role in modulating expression of gluconeogenic genes during fasting [40]. Furthermore, it has been shown that FOXO1 impairs both glycolysis and mitochondrial respiration by antagonizing MYC signaling [41]. These findings raise the question whether B cell identity is linked to a specific metabolic state. Using pharmacological as well as genetic approaches, Adams et al. demonstrated that plasma cell differentiation is coupled with glycolysis, and that self-renewal of B cells is maintained by oxidative phosphorylation [42]. For instance, inhibiting glycolysis using 2-Deoxy-D-glucose (2-DG) stimulated self-renewal, while using oligomycin to inhibit oxidative phosphorylation shifted cell fate toward plasma cell differentiation [42]. Dynamin-related protein 1 (Drp1) is essential for mitochondrial dynamics by forming helices around mitochondria to modulate mitochondrial division [43]. Interestingly, overexpression of the dominant negative mutant (Drp1^{K38A}) or treatment with a pharmacological inhibitor of Drp1 (mDivi−1) resulted in increased plasma cell differentiation [42], suggesting that mitochondrial fission and its impact on energy metabolism may play a role in determining B cell fate.

A B-lymphoid transcriptional program that negatively regulates glucose uptake and energy metabolism

More recently, our group studied whether B-lymphoid transcription factors can regulate glucose and energy supply in B-cell precursor cells, and whether this has implications for transformation and leukemogenesis [18]. Combining gene expression and ChIP-seq analyses, a novel B-lymphoid program for transcriptional repression of glucose uptake/utilization *(INSR, GLUT 1/3/6, HK2/3* and *G6PD)* and activation of inhibitors of glucose transport, including *NR3C1* [glucocorticoid receptor; 44], *TXNIP* [glucose feedback sensor; 45] and *CNR2* [cannabinoid receptor; 46] has been identified [18]. Consistent with changes in gene expression observed, inducible reconstitution of PAX5 or IKZF1 in patient-derived pre-B ALL cells lacking functional PAX5 or IKZF1 resulted in a state of chronic energy deficit. Reconstitution of PAX5 or IKZF1 resulted in decreased glucose uptake and ATP levels, leading to activation of the energy stress sensor LKB1-AMPK [18]. B→ myeloid reprogramming mediated by the myeloid transcription factor CEBPa subverts B-lineage commitment by repression of *PAX5* [18,22]. Notably, gene expression analysis revealed transcriptional activation of multiple mediators of glucose uptake/metabolism and energy supply *(Insr, Glut1, Glut6, Hk3, Pygl* and *G6pd)* upon B→ myeloid reprogramming [22]. Likewise, reversal of B-lymphoid lineage commitment relieves restriction of glucose and energy supply. B→myeloid reprogramming was shown to salvage chronic energy deprivation and restore a state of energy abundance characterized by increased glucose uptake and cellular ATP levels [18].

Mechanistic contribution of PAX5-targets to negative regulation of glucose metabolism

CRISPR/Cas9-based genetic screens identified NR3C1, TXNIP and CNR2 as central effectors of the tumor suppressive function of PAX5 [18]. Importantly, loss of *Nr3c1* [47], *Txnip* [48] or *Cnr2* [49] function increased both glucose uptake and cellular ATP levels in murine pre-B ALL cells, validating specific aspects of regulation of glucose uptake and energy metabolism by these molecules [18]. Furthermore, agonists of TXNIP and CNR2 synergized with GCs to induce cell death of patient-derived pre-B ALL cells, exacerbating ATP deficit in pre-B cells [18]. Taken together, these findings suggest that B-lymphoid transcription factors (PAX5 and IKZF1) restrict glucose uptake, which is upstream of both glycolysis and oxidative phosphorylation, limiting glucose and energy supply to levels not sufficient for transformation of pre-B cells. Therefore, B-lymphoid transcription factors PAX5 and IKZF1 function as metabolic gatekeepers.

Transport-independent pyruvate- and TCA cycle metabolites can bypass the gatekeeper function of PAX5 and IKZF1

During early B cell development, pre-B cells undergo intense selective pressure and sustain DNA damage during multiple rounds of V(D)J recombination of immunoglobulin heavy and light chain genes [50]. Acquisition of genetic lesions during the V(D)J recombination process at the pre-B cell stage represents a major driver of clonal evolution [51,52], which is

compounded by the high rate of proliferation of pre-B cells. Given the inherent risk of malignant transformation during early B cell development, the B-lymphoid transcriptional program for the restriction of glucose and energy supply may function as an additional safeguard against malignant transformation. Indeed, transport-independent lipophilic methyl-conjugates of pyruvate and TCA cycle intermediates (oxaloacetate and dimethyl succinate) can bypass the tumor suppressor function of PAX5 and IKZF1, jumpstarting oncogenic signaling and enabling leukemic transformation of pre-B cells [18; FIGURE 1].

Latent persistence of pre-leukemic B cell clones during early childhood and in healthy adults

Under this scenario, one would predict that healthy individuals frequently carry pre-malignant B cells that harbor an oncogenic lesion in the presence of fully functional PAX5 and IKZF1. Indeed, pre-leukemic B cell clones carrying multiple oncogenic lesions *BCR-ABL1* [31], *MLL-AF4* [53], *ETV6-RUNX1* [54] gene rearrangements and hyperdiploidy [55] are frequently found in cord blood samples from healthy neonates. Furthermore, silent oncogenes including *BCR-ABL1* [56,57] and mutant *RAS* [58] are often found in small fractions of normal B cells in healthy adults. In the bone marrow of asymptomatic CML patients in remission, ~10% of pre-B cells carry a silent *BCR-ABL1* oncogene [59]. In patients with Noonan syndrome and RAS-associated lymphoproliferative disorder (RALD), *RAS* pathway mutations cause pre-B cell expansions but give rise to leukemia only in very few cases [58]. These findings collectively suggest that pre-leukemic B cell clones frequently occur in healthy individuals, during early childhood and in healthy adults. The high frequency of genetic lesions observed in B-lymphoid transcription factors in pre-B ALL takes on new significance from the finding that B-lymphoid transcriptional program limits glucose and energy supply, and thus functions as a metabolic barrier against oncogenic transformation [18].

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Highlights

- Glucocorticoid activity in leukemia depends on B-lymphoid transcription factors.
- B cell identity is marked by chronic energy deficit.
- PAX5 and IKZF1 restrict glucose uptake and energy metabolism.
- PAX5 and IKZF1 safeguard against leukemic transformation of B cell precursors.

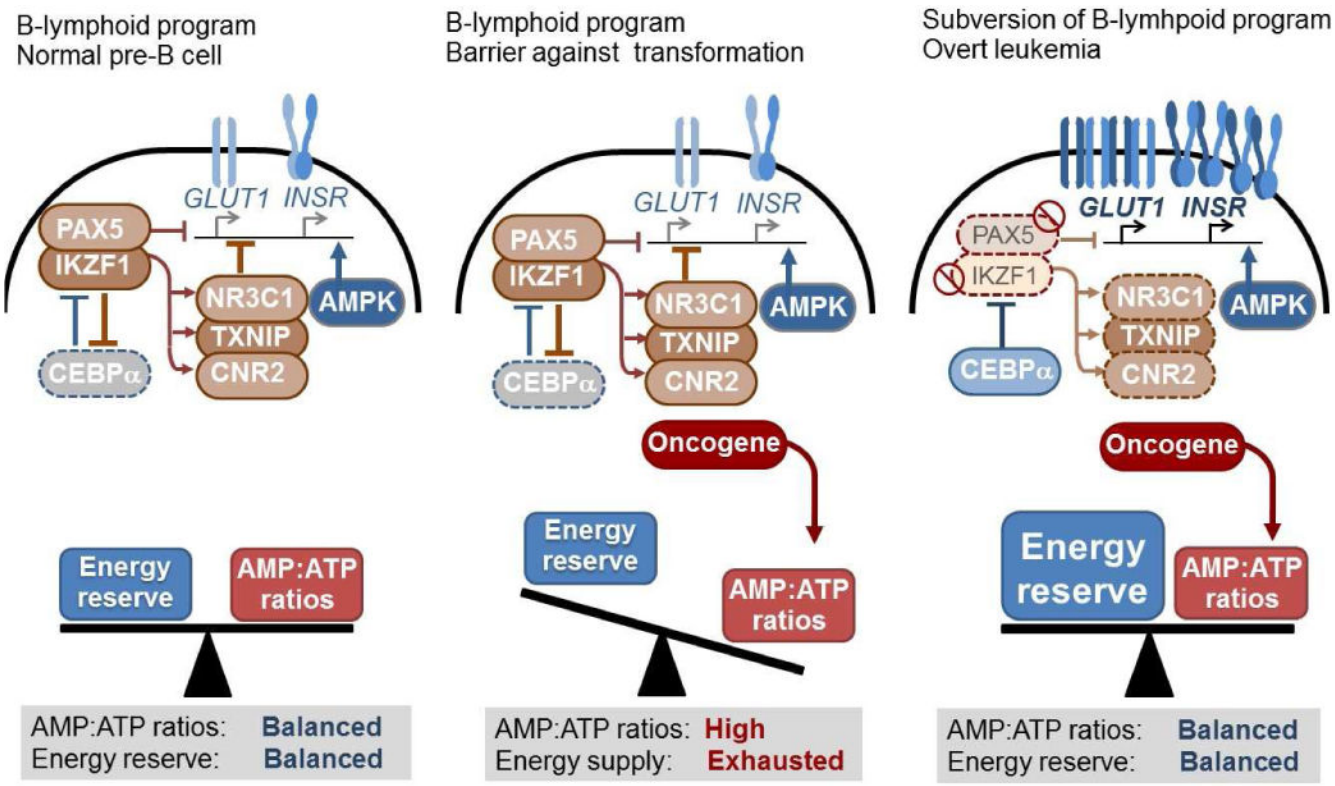

Figure 1. Metabolic gatekeeper function of B-lymphoid transcription factors

B-lymphoid transcription factors (e.g. *PAX5* and *IKZF1)* are essential for early B cell development, and they oppose myeloid differentiation as an alternative lineage fate through repression of the myeloid transcription factor *CEBPα* and *vice versa.* While often transformed by the same oncogenes, B-lineage and myeloid leukemias are distinct with respect to their biological and clinical characteristics. In contrast to myeloid leukemia cells, pre-B ALL cells have high AMP:ATP ratios indicative of a state of chronic energy deficit, resulting in the activation of the energy-stress sensor AMPK. Transforming oncogenes (e.g. *BCR-ABL1, RAS)* impose significant metabolic requirements on ATP supply. In addition to their function in B cell development, B-lymphoid transcription factors function as a metabolic barrier against malignant transformation. Genetic lesions in B-lymphoid transcription factors represent near-obligate defects in human pre-B ALL. B-lymphoid transcriptional program represses glucose uptake as well as utilization (e.g. *GLUT1, INSR),* while activating inhibitors of glucose transport *(NR3C1, TXNIP* and *CNR2).* B-lymphoid lineage commitment limits the amount of glucose and energy supply to levels that are not sufficient for leukemic transformation. Genetic deletion of *NR3C1, TXNIP and CNR2* revealed these inhibitors of glucose transport as key effects of PAX5-mediated tumor suppression.

Table 1

Comparison of biological characteristics of myeloid and B-lineage leukemia cells

Characteristic	Myeloid	B-lineage
Transforming oncogenes	*RAS* (AML) *BCR-ABL1* (CML)	*RAS* *BCR-ABL1* (*Ph*$^+$ ALL)
Lesions in transcription factors	*CEBPA* (5–14%; AML)[60], *GATA2* (11%; CML-blast crisis)[61], *PU.1* (7%; AML)[62]	*IKZF1* (84% of *Ph*$^+$ ALL cases), *EBF1* (14%), *PAX5* (51%)[63]
AMP:ATP ratio	0.06 ± 0.03 (CML)[18]	4.5 ± 1.2 (*Ph*$^+$ ALL)
Doubling time	11 – 14 days (AML)[64] 48 days (CML)[65]	2 days[66]
Cytoplasmic volume (μm^3)	739[67]	121[68]
Number of mitochondrial per cell	15.9 ± 3.8 (AML)[69]	7.8 ± 3.7[69]
Mitochondrial volume (μm^3)	42.9 ± 6.7[67]	15.0 ± 5.9[68]