IKKβ and NF-κB Transcription Governs Lymphoma Cell Survival through AKT-Induced Plasma Membrane Trafficking of GLUT1

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Abstract

All cancer cells require increased nutrient uptake to support proliferation. In this study, we investigated the signals that govern glucose uptake in B-cell lymphomas and determined that the inhibitor of NF-κB-kinase β (IKKβ) induced glucose transporter-1 (GLUT1) membrane trafficking in both viral and spontaneous B-cell lymphomas. IKKβ induced AKT activity, whereas IKKβ-driven NF-κB transcription was required for GLUT1 surface localization downstream of AKT. Activated NF-κB promoted AKT-mediated phosphorylation of the GLUT1 regulator, AKT substrate of 160kD (AS160), but was not required for AKT phosphorylation of the mTOR regulator Tuberous Sclerosis 2 (TSC2). In Epstein-Barr virus–transformed B cells, NF-κB inhibition repressed glucose uptake and induced caspase-independent cell death associated with autophagy. After NF-κB inhibition, an alternate carbon source ameliorated both autophagy and cell death, whereas autophagy inhibitors specifically accelerated cell death. Taken together, the results indicate that NF-κB signaling establishes a metabolic program supporting proliferation and apoptosis resistance by driving glucose import. Cancer Res; 71(23); 1–10. ©2011 AACR.

Introduction

Protooncogenes such as c-myc, Ras, and PI3K or inactivation of tumor suppressors such as PTEN and p53 are associated with alterations in cellular metabolism commonly referred to as the Warburg effect (1). Glucose consumption, a hallmark of the Warburg effect (2–5), is shared by many B-lymphomas and most antigen- or mitogen-stimulated lymphocytes, indicating the existence of a common regulatory mechanism to support rapid lymphocyte proliferation. NF-κB activation is a common feature of transformed B lymphocytes, such as Herpes virus-transformed lymphoblasts, multiple myeloma, and diffuse large B-cell lymphomas (DLBCL), and also of mitogen stimulation or antigen coreceptor signaling in B lymphocytes (6–9). For example, the toll-like receptor (TLR)-4, TLR-9, CD40, and BAFF-R engagement, as well as p53 depletion, were all shown to activate NF-κB signaling and stimulate glucose consumption (10–12). We hypothesized that the NF-κB pathway plays a critical role in glucose import.

NF-κB transcription factors are latent in the cytoplasm until activated in response to upstream signals that converge upon the inhibitor of NF-κB kinase (IKK) complex comprising IKKγ, IKKα, and IKKβ. IKKβ phosphorylates IkBα, thereby targeting it for proteasomal degradation, and allowing NF-κB to translocate to the nucleus. Noncanonical stimuli activate IKKα to phosphorylate p100 and induce p100 processing to p52 and its subsequent translocation to the nucleus (9). Some stimuli stabilize Bcl-3 and its binding to p50 or p52 homodimers to turn these repressive complexes into transcriptional activators (13).

Glucose import across the cell membrane is mostly facilitated by glucose transporters (GLUT; ref. 14). GLUT levels and activity are highly regulated by oncogenes and tumor suppressors. c-myc and Ras induce GLUT1 mRNA (15, 16), whereas p53 suppresses expression of GLUT1, GLUT3, and GLUT4 mRNA through hypoxia-inducible factor α (HIF1α; ref. 18) but also induces translocation of GLUT4 from storage vesicles to the plasma membrane (14). PI3K induces GLUT4 trafficking by activating AKT that, in turn, phosphorylates AS160. AS160 phosphorylation inhibits its GTPase activating protein (GAP) function toward Rab proteins, which in their GTP-bound form promote GLUT-vesicle movement to and fusion with the plasma membrane. Recently, the PI3K-AKT pathway was also implicated in the regulation of GLUT1 localization in T cells (19, 20).

In this study, we investigated the effects of IKKβ and NF-κB on glucose import and show that IKKβ and NF-κB

References


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transcription govern B-lymphoblast survival through AKT-induced GLUT1 plasma membrane trafficking.

Materials and Methods

Cell culture

wtLCL23, a spontaneous lymphoblastoid cell line (LCL) generated in the laboratory, and IB4tetANkBz α EBV+ LCLs (6), BLetLMP1 (21), and the DLBCLs, SUDHL1 and SUDHL6 (22) were cultured in RPMI (GIBCO) supplemented with 2 mmol/L glutamine and 10% (v/v) Fetalplex (Gemini Bio-products). BC3, BCBL, and BCLM (KSHV+ PEI; refs. 23–25) were cultured in RPMI supplemented with 2 mmol/L glutamine and 20% (v/v) Fetalplex. BLetLMP1 and IB4tetANkBz α were supplemented with 1 μg/mL tetracycline, 48H (GIBCO; 0.5 mg/mL) and hygromycin (EMD; 1:1,000). Cells harboring PGKop-based vectors were cultured in blasticidin (Invitrogen; 1 μg/mL). All cell lines were verified by viral gene expression and/or human CD19 expression and human CD54 expression. Cells were confirmed to be Mycoplasma negative by MycoAlert (Lonza).

Vectors

PGKbla was created by ligating a Bgl2-EcoRI fragment encompassing the NF-κB-insensitive PGK promoter from PGK2 vector (26) into Bgl2-EcoRI cut pcDNA6. PGKop was cloned by sequential ligation of EBNA1 as AatII/MfI and OriP as MfI fragments from pCEP4 into PGKbla cut with the same enzymes. EBNA1 and OriP, the Epstein-Barr virus (EBV) origin of replication, allow episomal maintenance of the plasmid. MyrAKT was cloned as a BamHI fragment from pBABEGFP-myrtAK into PGKbla and PGKop. The AKT S473D mutation was introduced by quick change (Stratagene) with the oligo-GAGCCTGA. GLUT1 with a 2-nucleotide TTCCCCCAGTTCGACTACTCAGCTAGCGGCA-

Surface GLUT1 assays

Cells were stained for 20 minutes at 4°C with a polyclonal rabbit anti-Flag antibody (1:200; Sigma) in FACS buffer (PBS, 0.1% SoAzide, and 5% serum). Cells were washed and labeled with Alexa Fluor–conjugated antibodies 1:200 in FACS buffer for 20 minutes at 4°C. Median fluorescence intensity of live cells (FSC/SSC gating) was determined by FACS and, if indicated, normalized to fGLUT1 over glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression. For transient assays, expression vectors were cotransfected with pEGFP-C1 (Clontech), and surface fGLUT1 levels were determined on GFP+ cells.
were cultured in RPMI with 10% serum and 50 μmol/L 2-NBDG. Median cell fluorescence was measured at multiple time-points between 5 and 32 minutes. The increase in fluorescence was linear and inhibited at 4°C. The slope of a linear regression was defined as the rate of glucose uptake and was normalized to the rate of 2-NBDG uptake of corresponding control cells. When indicated, phloretin (250 μmol/L) was added 15 minutes before and during the assay.

**Lactate assay**

Cells were washed 3 times and cultured for 4 hours in RPMI with 10% dialyzed serum (dialyzed 2 times, with molecular weight cutoff (MWCO) 3,500 Da, against 100× volume of PBS, for 24 hours). Lactate levels in the cell supernatants were measured with a lactate assay (Biovision) according to the manufacturer's instructions and normalized to the cell concentration.

**Immunofluorescence**

Concentrated cells were transferred in RPMI onto poly-d-lysine coated coverslips for 15 minutes, fixed with 50% methanol and 50% acetone for 2 minutes, blocked with RPMI/10% serum for 30 minutes, and incubated in primary antibody in RPMI/10% serum overnight. Cells were washed, incubated with species-specific Alexa Fluor–conjugated antibodies, and stained with TO-PRO3 DNA stain (1:400; Invitrogen). Slides were mounted with ProLong Antifade solution (Invitrogen) and images were captured with a Nikon PCM2000 coupled to a Zeiss inverted fluorescence microscope using Simple 32 software. GLUT1, GLUT3, HA, and LC3 staining was individually adjusted in Adobe Photoshop for maximal brightness. All LMP1 images in a single panel were acquired with the same exposure time and the brightness was adjusted identically. Nuclei staining (brightness and gamma) was corrected for optimal visualization.

**Immunoprecipitation**

Cells were lysed for 20 minutes in ice-cold immunoprecipitation (IP) buffer [20 mmol/L Tris pH 7.5, 50 mmol/L NaCl, 1 mmol/L EDTA, 1% NP-40, 2.5 mmol/L Na pyrophosphate, 1 mmol/L β-glycerophosphate, 1 mmol/L Na3VO4, 1% protease inhibitor cocktail (Sigma), and 1mmol/L PMSF]. AS160 was immunoprecipitated with 1 μg anti-AS160 antibody and 20 μL sepharose A beads (Invitrogen) with rotation at 4°C for 4 hours from cleared supernatants (10,000 rpm for 5 minutes).

**Statistical analysis**

Statistical differences were determined with a 2-tailed paired Student t test. P values are indicated.

**Results**

**IKKβ induces GLUT1-dependent glucose import**

To examine glucose import, we monitored the uptake of a fluorescent 2-deoxyglucose analogue (2-NBDG; ref. 28) in response to signals from the NF-κB activators EBV oncoprotein Latent Membrane Protein 1 (LMP1), LPS, or Cpg, in the NF-κBlow Burkitt lymphoma cell line BL41 that was stably transfected with LMP1 under tetracycline control (BLtetLMP1). All stimuli independently increased the rate of glucose uptake (Fig. 1A), but failed to do so in the presence of chemical IKKβ inhibitors (IKKβi or IKKβiβ) that specifically blocked canonical signaling (Supplementary Fig. S1A and S1B). Supernatant transfer from LMP1+ to LMP1− cells did not induce glucose import to the same extent, indicating that NF-κB regulation of glucose import is cell-intrinsic and not attributable to elevated cytokine signaling (Supplementary Fig. S1C). Phloretin, a specific GLUT inhibitor, blocked LMP1-induced glucose import (Supplementary Fig. S1D) indicating that LMP1-mediated NF-κB effects were dependent on proteins from the GLUT family. Therefore, we evaluated expression levels and localization of the predominant lymphoid GLUT family members, GLUT1 and GLUT3 (21, 29). LMP1 and LPS induced the NF-κB target TRAF1, and IKKβ prevented TRAF1 induction (Fig. 1B and C). Perturbation of the NF-κB pathway had no impact on GLUT1, GLUT3, or their transcriptional regulators HIF1α or c-myc. (ref. 15, 18; Fig. 1B and C; Supplementary Fig. S1E).

**IKKβ induces GLUT1 membrane localization**

Although GLUT abundance was not affected by IKKβ activation, we observed clear regulation of GLUT1 localization. In response to EBV LMP1, LPS, and Cpg, GLUT1 translocated from intracellular vesicles to the plasma membrane (Fig. 1D). In contrast, GLUT3 localized to cytosolic punctate independent of LMP1 expression (Supplementary Fig. S1F). In agreement with the glucose-import assays, IKKβ blocked the ability of all 3 independent stimuli to promote GLUT1 plasma membrane localization (Fig. 1D). To quantify the impact of IKKβ activation on GLUT1 plasma membrane levels, we stably expressed GLUT1 modified with a 2× Flag tag (fGLUT1) in the first extracellular loop (19) in BLtetLMP1 (BLtetLMP1–fGLUT1). LMP1 and LPS significantly increased surface fGLUT1 (Supplementary Fig. S1G–S1K) independent of GLUT1 expression levels (Supplementary Fig. S1K–S1M). This effect was dependent on IKKβ activity (Supplementary Fig. S1H–S1M). Furthermore, IKKβ treatment caused GLUT1 retention in wild-type LCL, Kaposi's sarcoma Herpes virus (KSHV)-infected peripheral effusion lymphomas (PEL), and DLBCL, showing that IKKβ governs GLUT1 localization in many B-cell malignancies (Fig. 1E; Supplementary Fig. S1N).

**IKKβ and PI3K are required for AKT activation**

GLUT1 plasma membrane localization in lymphocytes is regulated in a manner similar to that of GLUT4 in adipocytes, where GLUT4 translocates to the plasma membrane in response to insulin-induced PI3K and AKT activation (14, 19). Therefore, we sought to determine whether GLUT1 trafficking in response to NF-κB stimuli is AKT dependent. Similar to IKKβ inhibitors, the PI3K inhibitor LY294002 prevented LMP1-, LPS-, and Cpg-induced GLUT1 translocation and glucose import (Fig. 2A and B). Furthermore, PI3K inhibition by Wortmannin and LY294002 or AKT inhibition by an AKT inhibitor (Akti) led to retention of endogenous GLUT1 in wtLCL23, BCLM, and SUDHL4 lymphoma cells and fGLUT1.
in IB4-GLUT1 (Supplementary Fig. S2A–S2D). These data indicate that GLUT1 localization is PI3K, AKT, and IKK\(\beta\) dependent.

Because LMP1 and TLRs can activate AKT (30–32), we sought to determine if IKK\(\beta\) functions in the AKT pathway. Indeed, both PI3K and IKK\(\beta\) inhibitors blocked LMP1- and LPS-induced AKT activation (Fig. 2C and D). In fact, IKK\(\beta\)i reduced LMP1-induced AKT activity within 5 hours (Supplementary Fig. S2E). In contrast to LMP1 and LPS, serum-induced AKT activation was unaffected by IKK\(\beta\)i (Supplementary Fig. S2F), indicating that the role of IKK\(\beta\) does not extend to growth factor receptors and also showing the specificity of the IKK\(\beta\) inhibitor. The IKK\(\beta\)-related TANK-binding kinase 1 (TBK1) was shown to phosphorylate AKT at S473 (33) raising the possibility that IKK\(\beta\)i effects may be mediated by TBK1 inhibition. However, IKK\(\beta\)i specifically inhibited Sendai virus-induced IKK\(\beta\)-dependent RelA S536 phosphorylation with no effect on TBK1-dependent IRF3 dimerization (Supplementary Fig. S2G; ref. 34) and neither LMP1 nor LPS induced IRF3 dimerization in BLtetLMP1 (Supplementary Fig. S2H).

Because IKK\(\beta\)i caused GLUT1 retention in wtLCLs23, BCLM, and SUDHL4, we examined the effect of IKK\(\beta\)i on AKT activity in these cell lines. IKK\(\beta\)i only modestly reduced AKT S473 phosphorylation (Supplementary Fig. S2C), indicating that IKK\(\beta\) had a second effect on GLUT1 trafficking. This was supported by the observation that cycloheximide had no effect on LPS-induced AKT activation (Fig. 2D), but completely blocked LPS- or CpG-induced surface GLUT1 translocation and glucose import (Fig. 2A and B; Supplementary Fig. S1J). Thus, IKK\(\beta\) induces AKT that, in turn, is essential for GLUT1 plasma membrane accumulation. Nonetheless, AKT activation is not sufficient for GLUT1 plasma membrane targeting in the absence of continuous protein synthesis. We reasoned that NF-\(\kappa\)B-mediated gene expression may be necessary for IKK\(\beta\) stimuli to promote AKT-regulated GLUT1 localization.

**NF-\(\kappa\)B transcription supports GLUT1 membrane localization downstream of AKT**

To determine the requirement for NF-\(\kappa\)B transcription on GLUT1 localization and glucose import, NF-\(\kappa\)B complexes

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**Figure 1.** EBV LMP1, LPS, and CpG stimulate glucose import and promote GLUT1 plasma membrane localization via IKK\(\beta\). A, glucose uptake was measured by 2-NBDG fluorescence in BLtetLMP1 induced to express LMP1 (24 hours), stimulated with LPS (500 ng/mL; 10 hours), or stimulated with CpG (250 nmol/L; 10 hours). IKK\(\beta\)i (10 \(\mu\)mol/L) and DMSO were added at 9 hours (LMP1) or 0 hours (LPS/CpG). Percentage change of 2-NBDG uptake over control cells is presented (\(n = 4\), LMP1; \(n = 3\), LPS/CpG; mean ± SD; \(* P < 0.05; \,** P < 0.005\). B and C, GLUT1 and GLUT3 expression in total cell lysates from (A). TRAF1 and GAPDH expression serves as controls for NF-\(\kappa\)B activity and protein content. D, GLUT1 (green), DNA (blue), and where indicated, LMP1 (red) immunofluorescence of cells from (A). E, GLUT1 localization in IKK\(\beta\)i- or DMSO-treated SUDHL4 (9 hours), BCLM (9 hours), and wtLCL23 (24 hours) cells. Bar, 10 \(\mu\)m.
were retained in the cytoplasm by a tetracycline-inducible NF-κB super-repressor, DNIXBα, in the LMP1+ lymphoblastoid cell line IB4 (IB4tetΔNIXBα; ref. 11). NF-κB inhibition caused a loss of glucose import and surface endogenous- or flag-GLUT1 over 3 days (Fig. 3A and B; Supplementary Figs. S3A, S3B, and S1G) without impacting GLUT1 and GLUT3 expression or GLUT3 localization (Fig. 3C; Supplementary Fig. S1F). DNIXBmo modestly decreased AKT S473 phosphorylation without impacting AKT phosphorylation at the PDK1 site T308 or its activity toward an established target, TSC2 (Supplementary Fig. S1F). DNIXBmo modestly decreased AKT S473 phosphorylation without impacting AKT phosphorylation at the PDK1 site T308 or its activity toward an established target, TSC2 (Supplementary Fig. S1F). DNIXBmo modestly decreased AKT S473 phosphorylation without impacting AKT phosphorylation at the PDK1 site T308 or its activity toward an established target, TSC2 (Supplementary Fig. S1F).

NF-κB transcription is essential for AKT-mediated AS160 phosphorylation

AKT promotes GLUT4 membrane localization by inhibitory phosphorylation of AKT substrate of 160 kDa (AS160; ref. 14). To analyze AS160 impact on GLUT1 localization in lymphocytes, we transfected IB4 or IB4ΔNIXBα-GLUT1

Figure 2. EBV LMP1-, LPS-, and CpG-stimulated GLUT1 plasma membrane localization is dependent on AKT activation and continuous translation. AKT activation is both IKKβ and PI3K dependent. A, GLUT1 localization (green), DNA staining (blue), and where indicated, LMP1 expression (red) in BLtetLMP1 expressing LMP1 (24 hours), or stimulated with LPS (500 ng/mL; 10 hours), or stimulated with CpG (250 nmol/L; 10 hours). DMSO, LY294002 (LY; 30 μmol/L), or cycloheximide (CHX; 10 μg/mL) were included at 6 (LMP1) or 0 hours (LPS/CpG) where indicated. Bar, 10 μm. B, glucose/2-NBDG import as in Fig. 1A (n = 3, mean ± SD, †, P < 0.05; ‡, P < 0.005). C, AKT activity in BLtetLMP1 induced to express LMP1 and treated with DMSO, IKKβ (10 μmol/L at 6 hours), or Wortmannin (Wo; 1 μmol/L at 22 hours). At 24 hours, total cell lysates were analyzed for LMP1, AKT, and AKT(pS473). TRAF1 serves as an indicator of NF-κB activity. D, AKT activity in uninduced BLtetLMP1 pretreated with IKKβ, CHX, or Wo for 15 minutes and then stimulated with LPS. Total cell lysates were prepared at the indicated times and analyzed for AKT and AKT(pS473).
with expression vectors for control, HA-AS160, or mutant HA-AS160 lacking all AKT phosphorylation sites (HA-AS160-4p; S318A, S588A, T642A, and S751A; ref. 27). HA-AS160 expression had no impact on GLUT1 localization, whereas HA-AS160-4p caused retention of both endogenous- and flag-GLUT1 (Fig. 4A and B). Thus, we inferred that AS160 is an essential regulator of GLUT1 membrane localization in B lymphocytes.

Consistent with constitutive GLUT1 localization at the plasma membrane, AS160 was phosphorylated at AKT sites (PAS) in IB4tetΔNixBx (Fig. 4C). Wortmannin inhibited AS160 PAS-phosphorylation in control uninduced cells, but had little effect in IB4tetΔNixBx with or without ΔNixBx expression (72 hours). Bar, 10 μm. C, GLUT1 and GLUT3 expression in total cell lysates from (B). TRAF1 expression is an indicator of NF-κB activity, Tubulin is a loading control. D, GLUT1 localization in vector-, myrAKT (mAKT)-, or myrAKTS473D (mAKTS473D)-transfected IB4tetΔNixBx after treatment with Wortmannin (Wo; 9 hours) or induction for ΔNixBx expression (72 hours). Bar, 10 μm. E, surface flag-GLUT1 in the indicated conditions 48 hours after transfection. IB4tetΔNixBx-flag-GLUT1 cells were transfected with expression vectors for GFP and control, myrAKT (mAKT), or myrAKTS473D (mAKTS473D). After 24 hours, ΔNixBx was induced in one fourth of the cells. The residual uninduced cells were treated with either DMSO or Wo (0.1 or 1 μmol/L) for the final 9 hours before determining surface flag-GLUT1 expression in GFP+ cells. Percentage change over vector-transfected, uninduced, and untreated cells is presented. (n = 3, mean ± SD; *P < 0.05; **P < 0.005).

on surface endogenous- or flag-GLUT1 (Fig. 4C; Supplementary Figs. S3F and S2B).

We discovered that NF-κB is specifically required to recruit AKT for the phosphorylation of AS160. Inhibition of NF-κB–mediated transcription by ΔNixBx resulted in loss of AS160 PAS-site phosphorylation in control, myrAKT−, and myrAKTS473D-expressing cells (Fig. 4D). Importantly, the effect of NF-κB was specific to AS160 as the AKT target TSC2 T1462 phosphorylation was unaffected by NF-κB inhibition (Fig. 4D). Moreover, the activity of AMP-activated protein kinase α (AMPKα), which can promote AS160 phosphorylation (21), was not altered after NF-κB inhibition (Supplementary Fig. S1E). Thus, we have shown that the NF-κB pathway has 2 roles in GLUT1 localization. IKKβ is required for AKT activation, whereas NF-κB–mediated transcription allows AKT to phosphorylate AS160 (Fig. 4E).
Carbon availability is a significant feature of NF-κB prosurvival signaling.

To assess the importance of NF-κB effects on GLUT1 and lymphoma cell metabolism, we used EBV-transformed lymphoblastoid cells. EBV transforms primary B cells into lymphoblastoid cells, without required somatic mutations, that are highly reliant on EBV LMP1-mediated NF-κB activation for proliferation and survival (6). LCLs die after NF-κB inhibition over the course of 1 week, and cell death is not abrogated by caspase inhibitors (ref. 6; Supplementary Fig. S4A). Because ΔNixBt reduced glucose import resulting in decreased lactate secretion (Fig. 5A), we evaluated whether reduced carbon availability contributed to LCL cell death after NF-κB inhibition. NF-κB–inhibited cells were cultured with additional substrates for the tricarboxylic acid (TCA) cycle. Increasing the initial glutamine concentration from 2 to 22 mM and adding 20 mM 2-oxoglutarate improved IB4tetΔNixBt survival from 40% to 59% after 5 days of ΔNixBt expression (Fig. 5B). Furthermore, NF-κB inhibition increased sensitivity to the respiratory chain inhibitor oligomycin even in the presence of caspase inhibitor N-(2-Quinolyl)valyl-aspartyl-(2,6-difluorophenoy)methyl Ketone (Q-VD), indicating that NF-κB inhibition renders LCLs more reliant on mitochondrial metabolism (Supplementary Fig. S4B and S4C). Macro autophagy (here autophagy) can be induced as a prosurvival mechanism during starvation to sustain ATP and carbon availability by degrading cytosolic components (37). As has been observed in other LCLs (38), uninduced IB4tetΔNixBt exhibited low levels of...
autophagy, as measured by LC3b foci (Fig. 5C). Three days after ΔNXβB treatment, we observed a dramatic accumulation of LC3b foci (Fig. 5C) and of autophagosome-associated, phosphatidylethanolamine-conjugated, LC3b (LC3b-Ⅱ) in the corresponding cell lysates (Fig. 5D). Both indicators of autophagy were reduced when cells were grown in high glutamine and α-ketoglutarate, indicating that ΔNXβB caused starvation that, in turn, induced autophagy (Fig. 5C and D). Interestingly, the autophagy inhibitors 3-methyladenine (Fig. 5E) or chloroquine (Supplementary Fig. S4D) accelerated LCL death in NF-κB-inhibited cells but had no effect on NF-κB active cells. Glutamine and α-ketoglutarate partially reversed the increased sensitivity to autophagy inhibitors (Fig. 5E; Supplementary Fig. S4D).

Discussion

To support macromolecule synthesis, proliferating cells need to elevate nutrient uptake. B cells use glucose as their predominant carbon source. In this article, we have provided novel evidence that the IKKβ/NF-κB pathway induces glucose import by supporting GLUT1 plasma membrane localization. IKKβ kinase activity and NF-κB transcription function by regulating GLUT1 trafficking at separate points in the AKT pathway (Fig. 4E). Furthermore, we show that stimulation of glucose transport is a significant feature of NF-κB prosurvival signaling.

IKKβ and PI3K activity are necessary for LMP1 and LPS to stimulate AKT. Furthermore, AKT activates the IKK complex (39), creating a feed-forward mechanism that potentiates AKT activity. Recently, the IKKβ-related kinase, TBK1, was shown to phosphorylate AKT at S473 (33), raising the possibility that IKKβ might directly phosphorylate AKT. However, IKKβ may phosphorylate any of the numerous proteins that are established modifiers of PI3K-dependent AKT activation.

The requirement for IKKβ in LMP1- and LPS-mediated AKT activation and GLUT1 plasma membrane localization contrasts with the effect of TNFα-mediated IKKβ activity on GLUT4 trafficking (40). In adipocytes, TNFα inhibits insulin-induced GLUT4 membrane translocation through IKKβ-mediated inhibitory phosphorylation of IRS1 at S312. This divergent role for IKKβ may arise from stimulus-dependent differences in IKKβ complex formation. TNFR1 activates IKKβ via RIP1 whereas LMP1 and TLRs activate IKKβ via TRAF6 (41–43). Potentially only RIP1–IKKβ complexes recruit and phosphorylate IRS1, whereas TRAF6–IKKβ complexes do not. Consistent with this idea, we could not detect IRS1 phosphorylation at S312 despite constitutive IKKβ activity in LCLs (data not shown).

In contrast to IKKβ kinase activity, NF-κB–mediated transcription modulated AKT substrate recognition. Nuclear translocation of NF-κB subunits is essential for AKT phosphorylation of AS160, but not TSC2. Thus, NF-κB inhibition uncouples AKT effects on glucose import from TORC1 activation and illustrates a novel mechanism of stimulus-dependent AKT substrate recognition. Although the identity of the transcriptional target(s) is unknown, we favor a simple model in which NF-κB drives transcription of a gene encoding a scaffold that allows AKT to interact with AS160. It is possible that such a scaffold also regulates additional AKT substrate recognition.

Figure 5. NF-κB–mediated transcription links carbon availability to cell survival in a model of NF-κB–dependent lymphoma. A, rate of lactate production of IB4tet/ΔNXβB expressing ΔNXβB at indicated time points. The percentage change over uninduced cells is presented (n = 3, mean ± SD; *, P < 0.05; **, P < 0.005). B, cell survival rate (% Annexin V, propidium iodide) of ΔNXβB-expressing cells grown in normal media with or without supplemental glutamine (L-Q; 22 mmol/L) and α-ketoglutarate (α-keto; 20 mmol/L). Day 5, n = 8, mean ± SD; **, P < 0.005. C, autophagosome marker LC3b localization (green) and DNA (blue) in ΔNXβB+ and ΔNXβB+IB4tet/ΔNXβB+ grown with QVD (10 μmol/L) with or without supplemental glutamine and α-ketoglutarate (72 hours). Bar, 10 μm. D, whole-cell lysates corresponding to (C) were analyzed for LC3b-Ⅰ and autophagosome-associated LC3b-Ⅱ expression. TRAF1 and GAPDH served as controls for NF-κB activity and loading, respectively. E, cell survival rate (high forward scatter, low side scatter) after autophagy inhibition with 3-methyladenine (3MA). IB4tet/ΔNXβB were cultured in ΔNXβB (black), ΔNXβB (white), or ΔNXβB+ with supplemental glutamine and α-keto (gray). After 48 hours, the indicated concentrations of 3MA were added for an additional 18 to 24 hours. Per cent cell survival was normalized to non-3MA–treated cells (n = 3, mean ± SD); ΔNXβB = 89%; ΔNXβB = 69%; ΔNXβB+ and L-Q/α-keto = 81%. F, model: impact of NF-κB transcription on metabolism.
Our results parallel the requirement for NF-κB and AKT in LMP1-induced migration in nasopharyngeal carcinomas and LMP1-induced lymphoma in transgenic mice (31, 44).

Tumor viruses such as EBV and KSHV evolved to exploit the normal signaling pathways that drive lymphocyte proliferation. In this study, we have shown that the EBV oncogene LMP1 and TLRs use the same IKKβ- and AKT-dependent mechanisms to stimulate glucose import. The importance of NF-κB–stimulated glucose import is evident as glutamine and α-ketoglutarate ameliorated the effects of NF-κB inhibition, including autophagosome formation, the dependence on autophagy, and cell death. These data support a model where NF-κB promotes survival of NF-κB–dependent lymphomas by ensuring ample glucose import for energy production and macromolecule synthesis. Autophagy is triggered through starvation after NF-κB inhibition to prolong survival by providing alternative substrates for metabolism (Fig. 5F).

It is not clear why 2 mmol/L glutamine (media concentration) was not sufficient to saturate glutamine metabolism. Recently, Wellen and colleagues have shown that hexosamines, predominantly derived from imported glucose, are necessary to transport glutamine (45). The supplementation of 22 mmol/L glutamine and 20 mmol/L α-ketoglutarate might be required to overcome decreased glutamine import secondary to decreased glucose import after NF-κB inhibition.

NF-κB inhibition sensitized lymphoblastoid cells to inhibitors of oxidative phosphorylation or autophagy. The combined targeting of NF-κB–mediated transcription and autophagy or mitochondrial metabolism is likely to be a highly effective chemotherapeutic strategy for lymphoma. NF-κB transcription is also essential in colorectal, breast, and lung cancer homeostasis and is thought to function through induced expression of antiapoptotic proteins (46, 47). However, many of these tumors have high GLUT1 expression (48, 49), which is important for cell survival and is associated with poor clinical prognosis (16, 50). Therefore, NF-κB may also contribute to enhanced survival in these tumors by facilitating AKT substrate interactions, GLUT1 membrane targeting, and glucose import.

Disclosure of Potential Conflicts of Interest

T.G. Sommermann was a 2008 GSK fellow. The other authors disclosed no potential conflicts of interest.

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