# Glycolytic rate and lymphomagenesis depend on PARP14, an ADP ribosyltransferase of the B aggressive lymphoma (BAL) family

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Poly(ADP-ribose)polymerase (PARP)14—a member of the B aggressive lymphoma (BAL) family of macrodomain-containing PARPs—is an ADP ribosyltransferase that interacts with Stat6, enhances induction of certain genes by IL-4, and is expressed in B lymphocytes. We now show that IL-4 enhancement of glycolysis in B cells requires PARP14 and that this process is central to a role of PARP14 in IL-4–induced survival. Thus, enhancements of AMP-activated protein kinase activity restored both IL-4–induced glycolytic activity in *Parp14*−/− B cells and prosurvival signaling by this cytokine. Suppression of apoptosis is central to B-lymphoid oncogenesis, and elevated macro-PARP expression has been correlated with lymphoma aggressiveness. Strikingly, PARP14 deficiency delayed B lymphomagenesis and reversed the block to B-cell maturation driven by the Myc oncogene. Collectively, these findings reveal links between a mammalian ADP ribosyltransferase, cytokine-regulated metabolic activity, and apoptosis; show that PARP14 influences Myc-induced oncogenesis; and suggest that the PARP14-dependent capacity to increase cellular metabolic rates may be an important determinant of lymphoma pathobiology.

**M**echanisms promoting cell survival are central to both physiological homeostasis and cancer. The immune system in particular depends on a reservoir of naïve lymphocytes in the $G_0$ stage of the cell cycle. Maintenance of this pool requires regulation of energy balance to promote cell survival while preserving a readiness to mobilize in response to a pathogen (1–3). Such maintenance depends on cytokines to trigger signal transduction pathways that prevent atrophy and subsequent apoptosis (4). Members of the cytokine group binding to hematopoietin receptors in the common γ-chain ($γ_c$) family, such as IL-4 and IL-7, play a crucial role in lymphocyte survival through signals downstream from Janus-family (Jak) kinases (5–7). Multiple classes of cytokines and growth factors initiate prosurvival signaling to regulate apoptosis in oncogenesis as well as immunity (1, 2). Tumors also are notable for cellular metabolic programs differing from their normal cellular counterparts (8, 9). These parallels between lymphocyte homeostasis and cancer cell physiology are underscored by findings indicating that the $γ_c$ cytokine IL-7 promotes lymphocyte survival in $G_0$ and energetics that favor this outcome, but when overexpressed can drive lymphoma formation in mice (1, 7, 10).

Among cytokines in the $γ_c$ family, interleukin-4 (IL-4) regulates vital cellular processes including lymphocyte differentiation, proliferation, and apoptosis (6). Moreover, IL-4 may parallel IL-7 in its ability to promote tumorigenesis (11), but IL-4 activates signaling components distinct from those induced by other cytokines in the $γ_c$ family. Thus, IL-4–induced activity of the same Jak kinases as IL-7 causes phosphorylation of a conserved tyrosine residue in an $N$-P-X-Y motif unique to the IL-4 receptor (6). This site recruits insulin receptor substrate-2 (IRS-2), which associates with the p85α regulatory subunit of phosphatidylinositol 3-kinase (PI3K) and leads to enhanced Akt kinase activity as well as apoptosis inhibition (6). Thus, IL-4 is thought to promote lymphocyte survival through an IRS-2-dependent PI3K-Akt pathway analogous to one used by growth factor receptors.

IL-4 also is unique among $γ_c$ cytokines in that it mobilizes Jak-mediated tyrosine phosphorylation of latent cytoplasmic Stat6 (signal transducer and activators of transcription 6) proteins in resting lymphocytes rather than Stat5 (5, 12). Tyrosine-phosphorylated Stat6 proteins dimerize, translocate to the nucleus, and bind to specific DNA sequences to regulate gene transcription (12). However, specific transcriptional cofactors and mechanisms used by Stat6 to mediate target gene regulation or specificity under physiological conditions are poorly understood. Furthermore, the extent to which Stat6 is vital for survival, and the mechanisms by which a Stat6 limb of IL-4–activated signaling pathways might function, remains to be elucidated.

Previous work identified a novel Stat6-interacting protein that influenced IL-4–induced gene expression (13). This partner of Stat6 encodes an ADP ribosyltransferase whose activity can participate in amplifying Stat6-mediated promoter induction (13–15). Mice lacking this protein, renamed PARP14 (13), contained normal numbers of B and T lymphocytes, but the proliferative response of anti-IgM-stimulated PARP14-null B cells to IL-4 was decreased, as was the induction of two genes that encode prosurvival proteins (15). These findings suggest that PARP14 is a coactivator of Stat6 at some target genes. Other work in a sarcoma cell line determined that human PARP14 regulates the ubiquitination and levels of phosphoglucose isomerase, a protein involved in intermediary metabolism (16). These latter results suggested a potential link between PARP14 and cellular metabolism. PARP14 and its impact on modifiers of B-lymphoid oncogenesis were particularly notable because PARP14 is overexpressed in mouse B-lymphoma cells and closely related to the human protein BAL/PARP9, heightened expression of which correlated with worse clinical outcome in patients with diffuse large B-cell lymphoma (DLBCL) (17).

We show here that IL-4 regulation of glycolysis and glucose oxidation requires PARP14. The metabolic state of the cells appears vitally linked to the prosurvival effects of IL-4. PARP14 is needed to achieve full prosurvival signaling by IL-4 in B cells. Inhibition of AMP-activated protein kinase (AMPK), a sensor of cellular energy status and metabolism (18), blocked IL-4–induced B-cell survival and glycolysis. Conversely, AMPK-activating interventions coordinately restored the glycolytic and prosurvival signaling of IL-4 in *Parp14*-null B cells. Strikingly, this macro-PARP facilitated B-lymphoid oncogenesis and alterations in developmental progression driven by c-Myc. Together, the data indicate that PARP14 accelerates lymphoma and regulates cellular energy metabolism to promote B-cell survival.

## Results

**PARP14 Mediates IL-4–Induced Protection Against Apoptosis.** Cytokines such as IL-4 inhibit the atrophy and subsequent apoptosis of lymphocytes removed from their normal microenvironment. There is evidence indicating that IL-4–induced Stat6 contributes to B-cell survival (19, 20). Starting after 9 h in short-term culture, wild-type (WT) B cells removed from their normal environment underwent substantial apoptosis that was decreased by IL-4 (Fig. 1*A* and Fig. S1*A–C*). This rescue was attenuated in both *Parp14⁻/⁻* and *Stat6⁻/⁻* B cells (Fig. 1 *A* and *B* and Fig. S1 *D* and *E*). In addition to Stat6 induction, IL-4 receptors signal through PI3K and the activating phosphorylation of Akt (5, 6). As expected, IL-4 treatment of WT B cells induced Akt phosphorylation at the T308 and S473 regulatory sites (Fig. 1*C* and Fig. S2). *Parp14⁻/⁻* and *Stat6⁻/⁻* B cells each showed normal Akt phosphorylation in response to IL-4. Akt acts in part by phosphorylating GSK3β and the transcription factors Foxo1 and 3a (21). Although PARP14 has been reported to influence protein stability (16), absolute levels and IL-4–induced phosphorylation of these downstream targets of Akt were intact in PARP14- and Stat6-null B cells (Fig. 1*D*). Thus, PARP14 mediates IL-4–induced rescue of B cells from apoptosis without apparent impact on Akt.

**PARP14 Is Essential for IL-4 Regulation of Glycolysis in B Cells.** Glucose metabolism plays a critical role in cell survival (22, 23), so glycolytic rates were assayed in B cells from WT, *Parp14⁻/⁻*, and *Stat6⁻/⁻* mice after culture in the presence or absence of IL-4. IL-4 increased rates of glycolysis in WT B cells, but this effect was impaired in B cells lacking either PARP14 or Stat6 (Fig. 2*A*). The impairment of IL-4–induced glycolysis in *Parp14⁻/⁻* B cells was not due to a global loss of their capacity to increase metabolism, as anti-IgM stimulation enhanced glycolysis in PARP14- and Stat6-null B cells to levels comparable to WT B cells (Fig. 2*B*). IL-4, which collaborates with B-cell receptor cross-linking to enhance proliferation (5, 6, 15, 24), increased the glycolytic response to anti-IgM in WT, but not PARP14- or Stat6-null, B cells (Fig. 2*B*). Indeed, the quantitative increase in glycolysis stimulated by IL-4 was greater in anti-IgM-stimulated B cells than in their resting counterparts (Fig. 2*C*). Glycolytic flux in T cells may be limited by transport of glucose, which can be

increased by the γc cytokine IL-7 (7). IL-4 treatment increased glucose uptake into B cells by a PARP14- and Stat6-dependent mechanism, whereas rates of lipopolysaccharide (LPS)-stimulated glucose uptake were comparable for B cells of each genotype (Fig. 2*D*). IL-7 stimulates increased glycolysis in and survival of T cells by pathways mediated in part by Stat5 (7). The cytokine BAFF promotes mature B-cell glycolysis and survival (25). However, neither PARP14 nor Stat6 influenced the effects of IL-7 or BAFF on B cells (Fig. 2*E* and Fig. S3). The dramatic IL-4 effect on glucose uptake likely reflects both the increased conversion of hexose through glycolytic demand and IL-4-induced increases in surface expression of transporters such as GLUT-1 that are PARP14-dependent (Fig. S4*A*). We infer that there are specific physiological roles for IL-4, Stat6, and PARP14 in metabolic regulation and survival distinct from the BAFF and IL-7-Stat5 pathways.

To test whether the defect of PARP14-null B cells in IL-4–induced glycolysis is intrinsic to hematopoietic cells, we performed bone marrow reconstitutions. PARP14 is too large for efficient retrovirion generation, but the middle (three tandem "macrodomains") and C-terminal (catalytic ADP ribosyltransferase) portions (PARP14-MC) sufficed for its transcriptional function (13, 14). Bone marrow cells lacking PARP14 were transduced with PARP14-MC cDNA or empty vector ("MiT"), and then used to reconstitute recipients (Fig. 3*A*). Glycolysis and apoptosis were measured in B cells derived from transduced (Thy1.1⁺) *Parp14⁻/⁻* marrow stem cells and compared with control samples. IL-4 did not increase glycolysis in B cells lacking PARP14 (MiT). In contrast, PARP14-MC-transduced B cells responded to IL-4 by enhanced glycolysis (Fig. 3*B*) and the ability of IL-4 to rescue B cells from apoptosis (Fig. 3*C*). We conclude that PARP14 plays a cell-intrinsic role in IL-4–regulated B-cell glycolysis and survival.

**Essential Role for PARP14 in Mediating Mitochondrial Activity in B Cells.** Mitochondrial stability and activity are crucial for cellular metabolism and apoptosis regulation after growth factor withdrawal. PARP14 mediates IL-4 induction of the prosurvival kinase Pim-1, which can impact energetics, and the mitochondrially associated Bcl-2-related protein Mcl-1 (15). IL-4 enhanced mitochondrial respiration and membrane potential in WT B cells, but *Parp14⁻/⁻* and *Stat6⁻/⁻* B cells did not demonstrate this response (Fig. 4 *A* and *B* and Fig. S4*B*). The glycolytic end product pyruvate can be metabolized to lactate or used by mitochondria after conversion to acetyl-CoA by pyruvate dehydrogenase (PDH). LPS stimulation increased lactate release by B cells but IL-4 did not, and lactate production by B cells of each genotype was comparable (Fig. 4*C*). In contrast, IL-4 accelerated glucose oxidation (conversion of the 6-position carbon of glucose into $CO_2$) by a PARP14- and Stat6-dependent mechanism (Fig. 4*D*). Consistent with this finding, PDH gene expression was lower in IL-4–stimulated lymphocytes lacking PARP14, and IL-4 increased expression of the citrate synthase gene in WT but not deficient B cells (Fig. 4*E*, Table S1, and Fig. S4*D*). This effect on two key steps leading into the Krebs cycle appeared not to be a global defect of mitochondrial biogenesis or energy generation: Genes such as Sdha were unaffected, and fatty acid oxidation in the B cells was neither stimulated by IL-4 nor dependent on PARP14 (Fig. 4*E* and Fig. S4 *C* and *E*). Thus, metabolism in B cells is coordinated after IL-4 stimulation so that a PARP14-dependent increase of glucose flux into pyruvate is used at least in part by greater mitochondrial respiration, with lactate production remaining constant.

**AMPK Activation Reverts Defects of IL-4–Treated *Parp14*-Null B Cells.** IL-4 enlarges resting B cells (24), so we tested its effect on biosynthesis that would increase ATP consumption. IL-4 increased protein synthesis in B cells within 3 h, whereas treatment with the protein synthesis inhibitor anisomycin blocked this effect (Fig. 5*A*). AMPK senses cellular energy status and signals increased glucose uptake, glycolysis, and mitochondrial activity
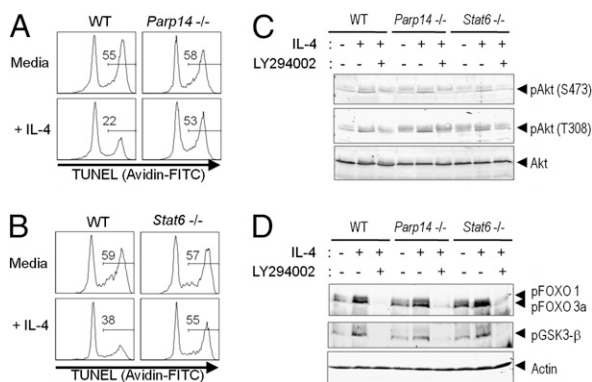


**Fig. 1.** IL-4 protection of B cells from apoptosis depends on PARP14. (*A* and *B*) PARP14 and Stat6 are vital for IL-4–induced suppression of B-cell apoptosis. Spleen cells [WT and knockout (KO) (*Parp14⁻/⁻* and *Stat6⁻/⁻*)] were cultured in the presence or absence of IL-4 and analyzed. Shown are FACS profiles of the TUNEL data (Avidin-FITC) from the B220⁺ gate in one experiment representative of five independent replicates. Numbers show the % of apoptotic events of the sample. (*C* and *D*) IL-4 activation of Akt signaling in *Parp14*- and *Stat6*-null B cells. Purified B cells (WT, *Parp14⁻/⁻*, and *Stat6⁻/⁻*) were stimulated with IL-4 (30 min) after pretreatment (30 min) with LY294002 as indicated, or cultured without IL-4. Shown are results of immunoblots using anti-pAkt (S473 or T308) and anti-Akt (*C*) or anti-pFOXO 1/3a and anti-pGSK-3β Ab along with anti-actin as a loading control (*D*). Data are from one representative result among three independent experiments.
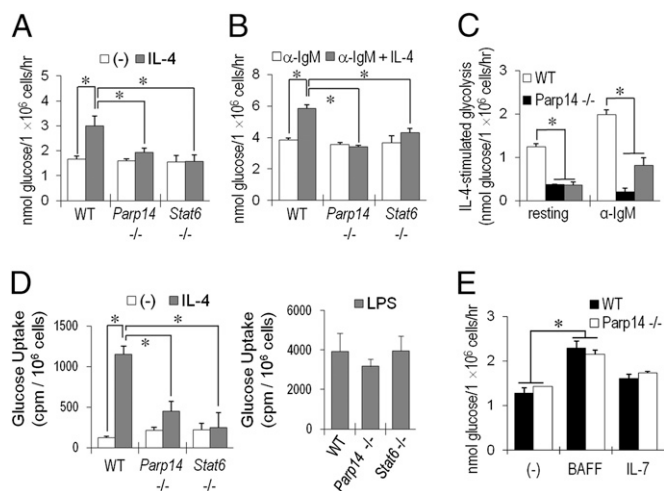
**Fig. 2.** IL-4 enhancement of glycolysis in B cells depends on PARP14. (*A*) After culture in the presence or absence of IL-4 (5 ng/mL), glycolysis assays were performed on equal numbers of surviving B cells from WT, *Parp14*-, or *Stat6*-null mice as detailed in *Materials and Methods*. (*B*) PARP14 and Stat6 influence glycolysis rates responsive to IL-4 treatment of activated B cells. As in *A*, except that cells were stimulated with anti-IgM (1 μg/mL) ± IL-4 (5 ng/mL). (*C*) Shown is a comparison of IL-4–stimulated glycolysis, calculated as [(glycolysis after IL-4 treatment) − (basal glycolysis)], for resting and anti-IgM–stimulated B cells. (*D*) IL-4–induced increase in glucose uptake is mediated by PARP14. Uptake was measured in equal numbers of WT, *Parp14*$^{-/-}$, and *Stat6*$^{-/-}$ viable B cells. (*E*) PARP14's role is distinct from IL-7 and BAFF. Glycolysis assays were performed with equal numbers of surviving cells after culture (20 h) of WT, *Parp14*$^{-/-}$, and *Stat6*$^{-/-}$ B cells in the presence or absence of IL-7 or BAFF. Shown are mean (±SEM) data from results in replicate experiments. Further data are in *SI Materials and Methods*.

(18). AMPK inhibitors (compound C or araA) reduced rates of protein synthesis in IL-4–treated cells to below those of unstimulated controls (Fig. 5*A*). AMPK inhibition either with compound C or a well-established dominant-negative mutant
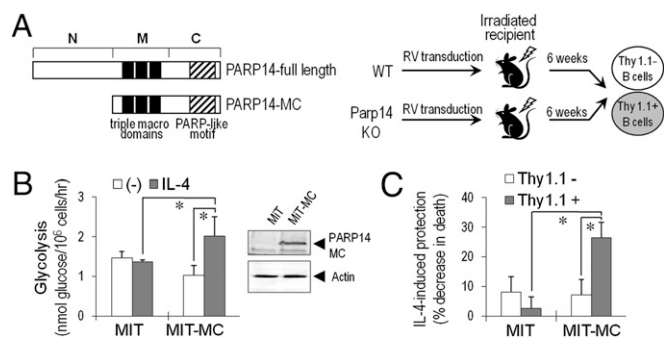


**Fig. 3.** Reversion of defects of PARP14-deficient B cells by hematopoietic cell transduction with *Parp14* cDNA. (*A*) Schematic of the experimental design and portion of PARP14 used for reconstitution. *Parp14*$^{-/-}$ bone marrow cells were transduced with empty (MIT) or PARP14-MC-expressing (MIT-MC) vectors and transferred into WT recipient mice. Glycolysis and apoptosis assays were performed on the purified Thy1$^+$ fraction of B cells harvested from these recipients. (*B* and *C*) Reconstitution with PARP14-MC enhances IL-4 induction of glycolysis (*B*) and mitigates the defect of IL-4–induced protection against apoptosis (*C*) in *Parp14*$^{-/-}$ B cells. CD3ε-negative Thy1.1$^+$ and Thy1.1$^-$ cells were isolated 6 wk postreconstitution and analyzed for glycolysis (*B*; as in Fig. 2) and apoptosis (*C*; as in Fig. 1). (*B*) Shown are mean (±SEM) IL-4–stimulated glycolysis assay results. (*Inset*) Immunoblot of PARP14-MC expression in transduced lymphoblasts. (*C*) B220$^+$ gated cells were analyzed by TUNEL assay; bar graphs show mean (±SEM) % apoptosis suppression by IL-4.

decreased glycolysis rates of IL-4–stimulated B cells to control levels and reduced IL-4 protection from apoptosis (Fig. 5 *B–E*). Thus, IL-4–induced B-cell survival required adequate AMPK activity and an ability to increase glycolytic metabolism.

To explore the relationship among IL-4, glycolysis, and AMPK, and to validate the effects of pharmacological agents used to probe the relationships between metabolism and antiapoptotic signaling by IL-4, we analyzed phosphorylation of AMPK and its substrate ACC. These analyses revealed that IL-4 induced p-AMPK(T172) but not p-ACC(S79), and activation of the pathway was effectively blocked by compound C (Fig. 5*F*). In contrast, inhibition of AMPK did not affect IL-4–induced p-Akt or p-Stat6 (Fig. S5 *B* and *C*). We further explored the relationship of PARP14 and the metabolic response to survival signaling by using the AMPK activator metformin (18) to test its impact on the defects observed in PARP14-deficient B cells. In the presence of IL-4, metformin reverted the glycolytic, mitochondrial, and survival defects of *Parp14*- and *Stat6*-null B cells (Fig. 5 *G* and *H* and Fig. S5*D*). Although metformin alone increased basal glycolysis rates in WT, *Parp14*, and *Stat6*$^{-/-}$ B cells slightly, IL-4 was essential for full recovery. Consistent with AMPK impacting the PARP14 role in glycolytic regulation, transduction of constitutively activated AMPK into anti-IgM–stimulated PARP14-deficient B cells largely reversed their defect of IL-4–enhanced glycolysis (Fig. 5*D*), paralleled by a similar impact on IL-4–induced survival (Fig. 5*E*). Thus, AMPK activation did not suffice to reverse defects in the absence of IL-4, but increased AMPK activity in IL-4–stimulated *Parp14*$^{-/-}$ B cells coordinately restored glycolysis, mitochondrial activity, and protection against apoptosis. Together with the results on AMPK inhibition, the findings indicate that an ADP ribosyltransferase, PARP14, helps to orchestrate a coordinate metabolic response with increased glucose flux through glycolysis and mitochondrial oxidative metabolism. This metabolic response is vital for the prosurvival signaling of IL-4.

**Absence of PARP14 Protects Against Myc-Induced Developmental Block and Lymphoma.** PARP14 is a member of the BAL family of macrodomain proteins implicated in the clinical aggressiveness of human B-cell lymphomas (17). Moreover, the capacity to reprogram metabolism may be central to oncogenesis, and suppression of apoptosis accelerates B-lymphoid malignancy (8, 9, 26). When we tested whether the absence of PARP14 from B cells influenced the prognosis for cancer-prone Eμ-*myc* transgenic mice (27), the absence of PARP14 delayed lymphomagenesis (Fig. 6*A*). Strikingly, PARP14 was required for the dramatic Myc-induced decrease in mature-phenotype B cells in premalignant marrow and deficit of follicular B cells in spleen (Fig. 6*B* and Table 1), although Myc in the absence of PARP14 induced pre-B lymphomas similar to the main phenotype of Eμ-*myc* tumors (Fig. S6). Myc expression in this system forces increased cell size on B-lineage cells (27, 28), but the absence of PARP14 from the B220$^+$ population countered this in each subset (Fig. 6*C* and Table S2). These effects were associated with decreased glycolysis in freshly isolated *Parp14*$^{-/-}$ B cells compared with controls and a quantitatively similar blunting of the glycolytic response to Eμ-*myc* in premalignant B-lineage cells lacking PARP14 compared with Eμ-*myc* controls (Fig. 6*D*). We further observed that survival signaling in *Parp14*$^{-/-}$ B220$^+$ Myc-transgenic cells was impaired compared with Eμ-*myc* controls (Fig. 6*E*), whereas the in vivo BrdU-labeling indices of Eμ-*myc*-transgenic B-lineage cells were unaffected by the lack of PARP14 (Fig. S7). These analyses of the premalignant bone marrow and spleen further highlight a striking mitigation of the impact of sustained Myc expression when PARP14 is absent. We conclude that expression of this B aggressive lymphoma family macro-PARP is vital for oncogenic and developmental effects of Myc as well as normal metabolic regulation of B cells.

## Discussion

A fundamental insight from this study pertains to the biological functions of ADP ribosyltransferases, in particular those recently
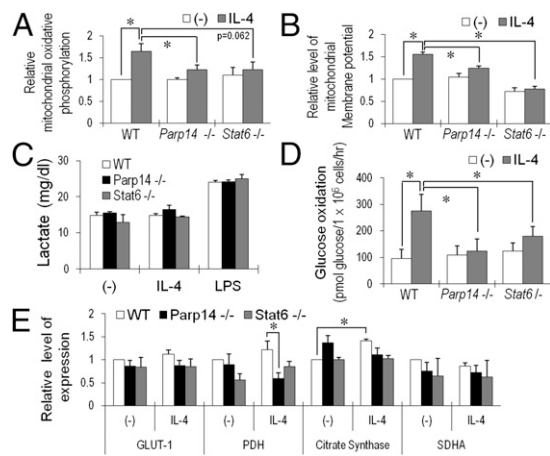
**Fig. 4.** PARP14 mediates IL-4–enhanced mitochondrial activity in B cells. (*A*) As detailed in *Materials and Methods*, mitochondrial oxidative phosphorylation was measured in B cells (WT, *Parp14*-, and *Stat6*-null) using a flow cytometric assay after culture (20 h) in medium ± IL-4. Specific mean fluorescent intensities (MFI) of mitochondrial signals for B220^{+} cells in matched samples of each independent experiment were normalized to the MFI of the WT sample cultured without added cytokine. Bar graphs represent the means (±SEM) of normalized values from these independent replicates. (*B*) PARP14 dependence of the IL-4–enhanced mitochondrial membrane potential in B cells. Mitochondrial membrane potential in samples as in *A* was measured using DiOC6 (3) dye; shown are mean (±SEM) data from independent replicate experiments. Signals for B220^{+} cells in matched samples of each independent experiment were normalized as in *A*. Bar graphs represent the means (±SEM) of three independent replicates. (*C*) Lactate in the medium was measured for purified B cells (WT, *Parp14*$^{-/-}$, and *Stat6*$^{-/-}$) after culture (20 h) in the presence or absence of IL-4 (5 ng/mL) or LPS (1 μg/mL). (*D*) PARP14- and Stat6-dependent enhancement of glucose oxidation by IL-4. Using B cells as in glycolysis assays, glucose oxidation was assayed as detailed in *Materials and Methods*. (*E*) PARP14 impacts PDH gene expression in IL-4–treated B cells. After first determining an optimal time for measuring induction by IL-4, RNA was isolated from B cells cultured for 4 h with and without IL-4; levels of mRNAs encoding metabolic genes were analyzed by quantitative real-time RT-PCR. Bar graphs show mean (±SEM) data from three independent experiments, with mRNA levels in each experiment normalized to WT B cells without IL-4 treatment and then averaged, as in *A* (*$P < 0.05$).



**Fig. 5.** PARP14, the metabolic response, and survival. (*A*) IL-4 increases protein synthesis in B cells. Mean (±SEM) incorporation of [$^{35}$S]methionine into WT B cells after a 3-h culture in the presence or absence of IL-4; where indicated, cells were pretreated 30 min with inhibitor. (*B* and *C*) AMPK inhibition attenuates glycolysis (*B*) and survival (*C*) of IL-4–treated B cells. Shown are the mean (±SEM) results of glycolysis and TUNEL assays of B cells pretreated with AMPK inhibitor (compound C; 1 μM) and then cultured ±IL-4. (*D*–*H*) AMPK activation in *Parp14*-null B cells reverses their defects of glycolysis and B-cell rescue by IL-4. (*D* and *E*) Activated B cells were transduced with MIT, MIT-AMPK-DN, or MIT-AMPK-CA retrovectors. Glycolysis (*D*) or apoptosis (*E*) of purified Thy1.1[+] (transduced) B cells was then assayed after culture ±IL-4 (20 h). (*F*) Activation of AMPK and ACC in WT and *Parp14*-null B cells. Purified B cells (WT and *Parp14*$^{-/-}$) were stimulated with IL-4 (2 h) or metformin, after pretreatment (30 min) with compound C as indicated. ΦNX cells treated with oligomycin (30 min) were used as a positive control. Shown are results of immunoblots using anti-pAMPK (T172) and anti-pACC (S79) along with anti-AMPK and anti-ACC as loading controls. (*G* and *H*) Effect of metformin on IL-4–enhanced glycolysis (*G*) and survival (*H*) of *Parp14* KO B cells. After 20 h culture in IL-4, metformin, or both, glycolysis and apoptosis were assayed; data shown are as in *B* and *C*.

identified as intracellular monotransferases (29). ADP ribosylation by endogenous activities of mammalian cells was first identified with PARP1, but 17–18 mammalian genes encode a module similar to the catalytic domain of PARP1 (30, 31). Unlike PARP1, PARP14 appears to catalyze mono- and not poly-ADP ribosylation (29). Furthermore, PARP14 is one of three members of the BAL family of PARPs containing macrodomains ("macro-PARPs") that bind (poly-)ADP ribose (32, 33). PARP1's roles in DNA repair, chromatin, and inflammation have been identified (30, 31), but the physiological functions of monotransferase or macro-PARP family members are not known.

Increased macro-PARP gene expression is associated with worse clinical outcomes of DLBCL (17), but there has been no direct evidence of an impact of these proteins on B-cell malignancy or of contributory mechanisms that could account for such a role. Our data indicate that PARP14 contributes to Myc-induced lymphoma pathobiology and provide evidence that PARP14 impacts two processes pertinent to B-lymphoid oncogenesis. First, PARP14 is vital for a coordinated metabolic effect whereby glycolysis and aspects of mitochondrial function are increased in a manner vital for IL-4–induced survival signaling of normal and premalignant Eμ-*myc*-expressing B-lineage cells. This role of PARP14 intersects with AMPK such that this central regulator of glycolysis coordinately restored glycolytic fitness and survival signaling when combined with IL-4. Second, this macro-PARP is vital for the impairment of developmental progression
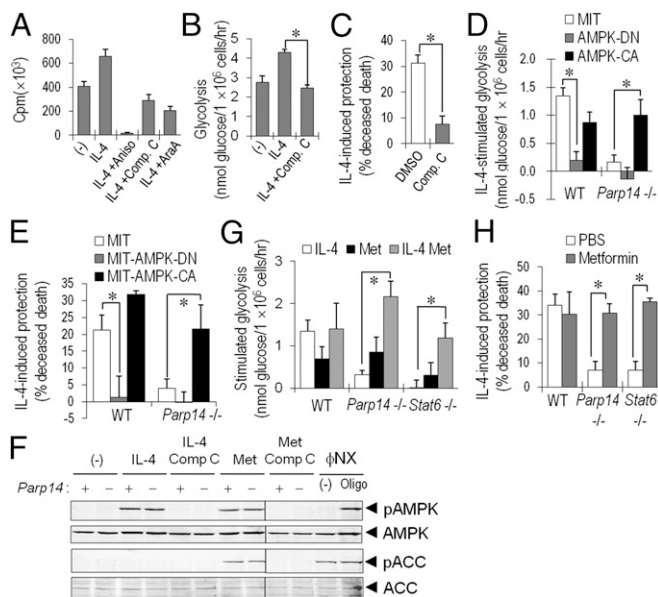
imposed by dysregulated c-Myc in the premalignant phase (27), so that the pool of target cells (pro-, pre-, and overall numbers of B lineage-committed cells) is expanded by Eμ-*myc* far less if PARP14 is absent. This effect, along with the lack of impact on IL-7-treated B cells, distinguishes PARP14 facilitation of Eμ-*myc* perturbations of B-lymphoid physiology from recent findings on an IL-7 receptor-Stat5 pathway important for c-Myc-driven lymphomagenesis (10, 34).

Reorganization of metabolism and a substantial increase in demands on the glycolytic pathway are features common among cancer cells, but the underlying molecular mechanisms effecting these changes are not clear. We found substantially increased glycolysis in premalignant B lineage-committed Eμ-*myc* bone marrow cells, consistent with the increased size of these cells and analyses of cell lines showing that c-Myc directly binds regulatory elements in the chromatin of genes encoding glycolytic enzymes to increase their expression (35). Of note, we found that PARP14 is crucial for Eμ-*myc* cells fully to increase their glycolysis and size. Thus, the proliferative drive and faster protein synthesis forced by sustained c-Myc overexpression (27, 28) must contend with a restriction on the rate of glycolysis when PARP14 is absent. Rapid engulfment of apoptotic cells in vivo precludes accurately measuring rates of apoptosis in situ. However, BrdU incorporation into all B220[+] subsets in vivo was similar in *Parp14*$^{+/+}$ and *Parp14*$^{-/-}$ Eμ-*myc* mice, and the ex vivo data collectively indicate that PARP14 is vital for sustaining normal
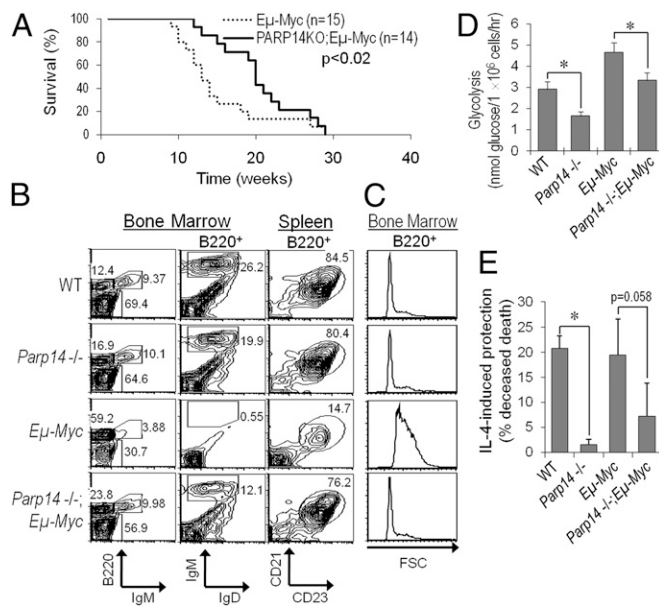
**Fig. 6.** PARP14 facilitates Myc-induced developmental distortion and lymphoma. (*A*) PARP14 deficiency protects against Myc-induced lymphoma. Survival curves for Eμ-*myc*, *Parp14* WT and Eμ-*myc*, *Parp14*$^{-/-}$ mice are shown. (*B*) PARP14 and the Myc-induced distortion of B-lymphoid development. B-lineage subsets in the premalignant bone marrow and spleen of mice (age 6 wk) were analyzed. Shown are results from one experiment representative of four independent replicates. (*C*) Myc depends on PARP14 for increasing cell size. Shown are forward scatter (FSC) profiles of B220$^+$ events. (*D*) Glycolysis in prelymphomatous Eμ-*myc*$^+$ B cells. Glycolysis was assayed in freshly purified B220$^+$ cells from WT, *Parp14* KO, Eμ-*myc*, and Eμ-*myc*, *Parp14*$^{-/-}$ mice (age 6 wk). Shown are mean (±SEM) data from three independent experiments (*$P < 0.05$). (*E*) PARP14 impacts IL-4 inhibition of Eμ-*myc*$^+$ B-cell apoptosis. Splenocytes from mice as in *D* were analyzed by TUNEL assay after culture in the presence or absence of IL-4. Bar graphs show mean (±SEM) IL-4–induced rescue from apoptosis in three independent replicate experiments.

glycolytic rates in vivo and mitigates the apoptotic stress of Myc. Perturbations that decrease apoptotic susceptibility commonly potentiate Myc-induced lymphomagenesis (26, 36). This suggests that the role of PARP14 in mediating regulation of B-cell metabolism and thereby enhancing survival is a significant component of its impact on oncogenesis.

The work also provides evidence of an interaction between AMPK activity and the prosurvival role of PARP14. The findings are intriguing in light of conflicting evidence from prior explorations of the relationship between AMPK and cell survival, and

evidence of a metformin effect on cancer in patients with diabetes (37), discussed further in *SI Discussion*. AMPK mediates a prosurvival signal initiated by adiponectin treatment of cardiomyocytes after ischemia and reperfusion and is similarly protective against neuronal apoptosis (38, 39). Conversely, sustained AMPK activation by high concentrations of an AMP analog caused apoptosis of normal and cancerous B cells and can similarly impact other cell types (40, 41). These opposite outcomes were due either to cell type-specific differences or the degree of AMPK activation, its duration, and cross-talk in which AMPK inhibits signaling via mTOR or exercises ACC-independent functions (42). In contrast to AMPK activation by stresses such as glucose deprivation, IL-4 stimulates mTOR (12). Of note, in the absence of IL-4, constitutively increased AMPK neither improved survival signaling nor raised rates of glycolysis to the level of IL-4–stimulated cells. Although the main role of AMPK may be to promote metabolic fitness on a pathway parallel to the IL-4–PARP14 mechanism, such findings indicate that coordination of at least two distinct mechanisms downstream from the IL-4 receptor is crucial. Akt activation by IL-4 was unaffected by the absence of PARP14, and this kinase can regulate survival by a mechanism that involves enhancement of glycolysis in coordination with mitochondrial stabilization (1, 22). Thus, although further study will be required to establish the nature of the PARP14-independent component(s), a contribution of Akt that cannot be replaced by AMPK is a straightforward model based on the data.

Finally, this work reveals that the capacity of Myc to impede B-cell maturation is strikingly dependent on PARP14. A block to B-lineage maturation in the bone marrow of Eμ-*myc*-transgenic mice has long been known (27), but the basis for this effect is not understood and conditional loss of *myc* also led to a deficit of mature B cells (43). Sustained c-Myc drives increased cell size along with persistent cell cycling and the attendant demand for increased energy production; the absence of PARP14 abrogated this increased size of B lineage-committed Eμ-*myc* cells. Developmental progression may require being able to exit the cell cycle (44), so it is tempting to speculate that the limitation in glycolysis prevented constant cycling, thereby allowing pre-B cells to mature. In addition, it is intriguing that bone marrow pre-B cells appeared more resistant to inhibition of glycolysis than their more mature IgM$^+$ progeny (45). In any case, the emergence of lymphoma is thought to be due in part to the accumulation of an expanded pre-B population in Eμ-*myc* mice (27, 28). Thus, the combined changes may account for the contribution of this BAL-family ADP ribosyltransferase to Myc-induced lymphomagenesis.

## Materials and Methods

**Table 1. PARP14 vital for Myc-induced hyper-cellularity and developmental distortions**

| | Bone marrow | | | | | | Spleen | |
| | B220+ cells | | Pre/proB (B220$^+$ IgM$^{lo}$) | | Mature B (B220$^+$ IgD$^+$ IgM$^{lo}$) | | FOB (B220$^+$ CD23$^+$ CD21$^+$) | |
|---|---|---|---|---|---|---|---|---|
| | % of total bone marrow cells | Number (×10$^5$)* | % among bone marrow cells | Number (×10$^5$) | % among bone marrow cells | Number (×10$^5$) | % of B220$^+$ spleen cells | Number (×10$^6$)$^†$ |
| WT | 30.3 ± 3.0$^‡$ | 13 ± 1.5$^‡$ | 18.4 ± 3.5 | 8.1 ± 2.2 | 13.3 ± 3.6 | 5.3 ± 1.2 | 75.9 ± 2.7 | 41.6 ± 15.6 |
| PARP14 KO | 35.5 ± 2.0 | 14 ± 0.7 | 20.4 ± 1.7 | 8.4 ± 1.3 | 13.4 ± 3.3 | 5.3 ± 1.3 | 77.1 ± 1.1 | 35.7 ± 12.7 |
| Eμ-*myc* | 55.6 ± 1.2 | 31 ± 2.5 | 48.1 ± 7.5 | 26.7 ± 4.6 | 0.8 ± 0.4 | 0.5 ± 0.2 | 15.7 ± 2.1 | 7.2 ± 3.2 |
| PARP14 KO/ Eμ-*myc* | 48.9 ± 9.4 | 20 ± 6.1 | 26.0 ± 2.7 | 10.6 ± 2.5 | 8.7 ± 1.2 | 3.2 ± 0.3 | 67.7 ± 3.7 | 28.2 ± 9.7 |

FOB, follicular B cells.
*(Number of recovered bone marrow cells) × (% in B220$^+$ gate).
$^†$(Number of recovered spleen cells) × (% in B220$^+$ CD23$^+$ CD21$^+$ gate).
$^‡$Mean ± SEM.

ventilated microisolators under specified pathogen-free conditions. Bone marrow cells were harvested after induction of donors with 5-fluorouracil, transduced with MiT vector or MiT-PARP14(MC) as described (13), and transferred into irradiated recipients (46). After 6 wk, B cells were isolated using magnetic beads, divided, cultured overnight ±IL-4, and assayed for apoptosis and glycolysis (modified by using $0.3 \times 10^6$ cells per sample for the triplicates). Further details are in *SI Materials and Methods*.

**Reagents, Cell Purification, Cytokines, Cell Culture, Flow Cytometry, and Apoptosis Assays.** Mouse B cells were purified (90–95%) by depleting Thy1^{+} cells from spleen cells using anti-Thy1.2, and these primary lymphocytes or bone marrow cells were cultured in the presence or absence of IL-4 as described (13). Further details and standard reagents from commercial suppliers are discussed in *SI Materials and Methods*. Published methods and commercial reagents detailed further in *SI Materials and Methods* were used for flow cytometric analyses including TUNEL assays (15).

**RNA and Protein Measurements.** Immunoblot and quantitative real-time RT-PCR analyses were performed using commercial antibodies and reagents by published methods (15) detailed further in *SI Materials and Methods*. Data are presented as values normalized to WT control and averaged over three independent experiments.

**Glucose Uptake, Glycolysis Assays, Measurement of Mitochondrial Parameters, and Lactate Production.** B cells ($10^6$ per sample) were cultured in the presence or absence of IL-4 or analyzed immediately after purification. As detailed in *SI Materials and Methods*, glycolysis in equal numbers of viable cells was then assayed by minor modifications of a standard technique measuring the conversion of 5-[$^3$H]glucose into diffused and undiffused $^3$HOH. Glucose uptake was measured using 2-[1,2-$^3$H]deoxyglucose after depleting intracellular glucose stores (details in *SI Materials and Methods*). As detailed in *SI Materials and Methods*, purified B cells recounted after culture were assayed by measuring rates of mitochondrial oxidation of fluorescent compounds or determination of $^{14}CO_2$ generation from [6-$^{14}$C]glucose. Lactate levels in the medium afterward were determined using a commercial kit.

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