Pellino 1 promotes lymphomagenesis by deregulating BCL6 polyubiquitination

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The signal-responsive E3 ubiquitin ligase pellino 1 (PELI1) regulates TLR and T cell receptor (TCR) signaling and contributes to the maintenance of autoimmunity; however, little is known about the consequence of mutations that result in upregulation of PELI1. Here, we developed transgenic mice that constitutively express human *PELI1* and determined that these mice have a shorter lifespan due to tumor formation. Constitutive expression of PELI1 resulted in ligand-independent hyperactivation of B cells and facilitated the development of a wide range of lymphoid tumors, with prominent B cell infiltration observed across multiple organs. PELI1 directly interacted with the oncoprotein B cell chronic lymphocytic leukemia (BCL6) and induced lysine 63–mediated BCL6 polyubiquitination. In samples from patients with diffuse large B cell lymphomas (DLBCLs), PELI1 expression levels positively correlated with BCL6 expression, and PELI1 overexpression was closely associated with poor prognosis in DLBCLs. Together, these results suggest that increased PELI1 expression and subsequent induction of BCL6 promotes lymphomagenesis and that this pathway may be a potential target for therapeutic strategies to treat B cell lymphomas.

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Pellino 1 promotes lymphomagenesis by deregulating BCL6 polyubiquitination

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The signal-responsive E3 ubiquitin ligase pellino 1 (PELI1) regulates TLR and T cell receptor (TCR) signaling and contributes to the maintenance of autoimmunity; however, little is known about the consequence of mutations that result in upregulation of PELI1. Here, we developed transgenic mice that constitutively express human PELI1 and determined that these mice have a shorter lifespan due to tumor formation. Constitutive expression of PELI1 resulted in ligand-independent hyperactivation of B cells and facilitated the development of a wide range of lymphoid tumors, with prominent B cell infiltration observed across multiple organs. PELI1 directly interacted with the oncoprotein B cell chronic lymphocytic leukemia (BCL6) and induced lysine 63–mediated BCL6 polyubiquitination. In samples from patients with diffuse large B cell lymphomas (DLBCLs), PELI1 expression levels positively correlated with BCL6 expression, and PELI1 overexpression was closely associated with poor prognosis in DLBCLs. Together, these results suggest that increased PELI1 expression and subsequent induction of BCL6 promotes lymphomagenesis and that this pathway may be a potential target for therapeutic strategies to treat B cell lymphomas.

Introduction
The pellino (PELI) protein family is highly conserved in the course of evolution and contains C3HC4 RING-like motifs in its C-terminal domains, which may serve as scaffold proteins (1). PELI proteins catalyze ubiquitin (UB) chains of several key molecules linked to lysine 48 (K48) or lysine 63 (K63) in B and T cell signaling, such as c-Rel and IL-1 receptor–associated kinase 1, respectively (2–5). Recent evidence from PELI1-deficient mice shows that PELI1 acts as a critical mediator of TRIF-dependent NF-κB activation in TLR3 and TLR4 pathways and is thus required for the induction of proinflammatory cytokine genes (2). Therefore, loss of PELI1 leads to hyperactivation and nuclear accumulation of c-Rel in response to T cell receptor–CD28 (TCR-CD28) signaling and facilitates the development of autoimmune diseases such as experimental autoimmune encephalomyelitis (6). In addition, evidence from PELI3-deficient mice reveals that PELI3 is not indispensable for the TLR-induced expression of proinflammatory cytokines and plays a negative regulatory role in TLR3– and virus-induced expression of type I IFNs and related genes (7). Overall, accumulated evidence suggests an important role for PELI proteins in regulating the proliferation and activation of B and T cells. However, their physiological roles remain unclear.

Activation of TCR-CD28–mediated signaling induces PELI1 expression (6, 8). In addition, TLR3 and TLR4 signaling activates the expression and E3 ligase activity of PELI proteins (7, 9). These observations suggest that PELI protein expression is strictly regulated by appropriate TCR or TLR signaling. Accordingly, expression of PELI proteins may be finely controlled, because their deregulation leads to diseases in murine models. Abrupt expression of these proteins may be closely associated with certain diseases, such as autoimmune diseases and cancer. Indeed, aberrant expression of receptor molecules in the immune system is frequently observed in many types of cancer in humans and is associated with cancer progression and poor outcomes (10, 11). Neoplastic and malignant B cells also show aberrant expression of receptor molecules such as TLRs (10). Notably, TLR3 and TLR4 are expressed by malignant B cells (10), which indicates that chronic active receptor-mediated signaling may facilitate the constitutive activation of PELI1 expression. In the present study, we demonstrated that PELI1 was overexpressed in numerous cells obtained from aggressive B cell lymphomas.

The transcriptional repressor BCL6 is highly expressed in germinal center (GC) B and T cells and is required for GC formation and antibody affinity maturation (12). Many B cell lymphomas originate at the GC of B cells and develop as a result of the deregulation of BCL6 expression; these include follicular lymphomas (FLs; almost 100%), Burkitt lymphomas (BLs; 100%), diffuse large B cell lymphomas (DLBCLs; >80%), and nodular lymphocyte-predominant Hodgkin lymphomas (>80%) (13). Notably, deregulation of BCL6 expression in lymphoid tumors...
occurs via some chromosomal rearrangement in 20%–40% of DLBCLs and 6%–14% of FLs (14, 15) and via some somatic mutation of the 5’-noncoding region of BCL6 in approximately 14% of DLBCLs (16). However, deregulation of BCL6 expression is not based solely on these genetic mutations. Recently, BCL6 has been found to be degraded by an SKP1-CUL1-F-box protein (SCF) Ub ligase complex containing the F-box protein FBXO11, but the FBXO11 protein is inactivated in DLBCLs (17). Therefore, the signaling pathway that regulates the ubiquitination of BCL6 may also contribute to B cell lymphomagenesis through BCL6 stabilization. However, little is known about the signals that stimulate commitment to B cells by activating BCL6 induction.

Results

PELI1 expression induces development of various lymphoid and solid tumors. To assess the gain of function of PELI1, we generated transgenic mice containing a human PELI1 gene coding sequence under the control of the β-actin promoter and the human early CMV enhancer. In these mice (referred to herein as PELI1-Tg), the PELI1 transgene was verified to be expressed in numerous organs, including the liver, lungs, BM, spleen, and thymus (Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI75667DS1). PELI1-Tg mice of 3 lines derived from founder C57BL/6-J mice showed accelerated mortality: median overall survival (OS) was about 560 days (~80 weeks), a significant reduction compared with non-Tg littermate control mice (P < 0.0001; Figure 1A). In addition, histopathological examination revealed that overall, 55% of adult PELI1-Tg mice developed a wide spectrum of tumors in the liver, lung, lymph nodes (LNs), pancreas, Peyer’s patch, spleen, and thymus (Figure 1, B and C). In particular, approximately 16%–20% of PELI1-Tg mice developed lymphoid tumors in spleen, thymus, LNs, and Peyer’s patches (Table 1). Most of the lymphoid organs of PELI1-Tg mice contained many cells showing plasmacytoid differentiation and some cells displaying histiocytic infiltration (Figure 1D). In addition to the high incidence of tumors in lymphoid organs, about 40% of PELI1-Tg mice also developed and showed different subtypes of tumors in liver, lung, intestine, pancreas, and prostate (Table 1 and Supplemental Table 1). Together, these results indicate that constitutive PELI1 expression contributes to the development of a wide range of tumors.

PELI1 expression induces constitutive activation of B cell signaling and tumor infiltration by B220+ lymphocytes in PELI1-Tg mice. Peripheral complete blood counts of PELI1-Tg mice showed significant increases in the number of white blood cells, lymphocytes, and monocytes (Supplemental Figure 2A), but there was no statistically meaningful sign of anemia compared with control non-Tg mice (data not shown). In addition, PELI1-Tg mice exhibited enhanced production of proinflammatory cytokines such as IL-6, IL-10, and TNF-α (Supplemental Figure 2B).

To further examine whether PELI1 overexpression would affect the aberrant proliferation and activation of B or T cells, or vice versa, lymphoid tissue samples were stained for B220 (proliferating B lymphocyte marker) and CD3 (T lymphocyte marker) antigens (Figure 2A). Flow cytometry showed that tumor-bearing lymphoid tissue samples of PELI1-Tg mice, including their LNs, spleen, and thymus, showed significantly increased proportions of B220+ lymphocytes compared with those in the same tissue of non-Tg mice of the same age (Figure 2A). The number of B220+ lymphocytes from the thymus was markedly larger in PELI1-Tg than non-Tg mice. In contrast, the number of CD3+ lymphocytes in the LNs, spleen, and thymus of some PELI1-Tg mice was smaller than that for non-Tg mice (Figure 2A).

TLR signaling is an upstream activating signal of PELI1 proteins and plays an important role in B cell activation. In response to TLR-mediated signals, B cells activate the expression of MHC class II and costimulatory CD86 molecules, which enhances their ability to activate helper T cells (18, 19). To further investigate the effect of PELI1 gain of function on receptor-mediated signaling, splenic B220+ cells were prepared from non-Tg and PELI1-Tg mice of the same age. Induction of CD86 expression as well as MHC class II expression was clearly evident in the absence of receptor-mediated stimulation with a CD40 or IgM antibody in PELI1-Tg mice (Figure 2B), which suggests that constitutive expression of PELI1 activated its downstream signaling without triggering the receptor-mediated signal. Furthermore, although stimulation of splenic B220+ cells using CD40 or IgM antibody led to induction of CD86 and MHC class II expression in both control non-Tg and PELI1-Tg mice, such expression was substantially augmented in the PELI1-Tg group compared with the non-Tg group (Figure 2B). Together, these results indicate that constitutive PELI1 expression induces ligand-independent B cell signal transduction.

Lymphocytemia persisted in most PELI1-Tg mice, which might be due to B220+ cell activation or proliferation. Therefore, tumor tissue samples were stained for B220, CD3, and CD23 antigens. Interestingly, histopathological examination of PELI1-Tg mice revealed markedly increased B220+ and CD20+ cells, but decreased CD3+ cells, in the spleen and thymus of PELI1-Tg mice, whereas staining for B220 and CD20 antigens was detectable only at the GC of non-Tg mice of the same age (Figure 2C). In addition, more than half of PELI1-Tg tumors showed lymphoid infiltration into multiple organs, including liver and lungs, which was represented by irregular nuclei, scanty cytoplasm, and a diffuse growth pattern (Figure 2D). The overall architecture of the lungs and liver of PELI1-Tg mice was distorted by atypical proliferating cells. To test whether these tumors were associated with a malignant lymphoid population derived from the PELI1 transgene, expression of B220, CD3, CD20, CD23, and Ki67 was assessed. Notably, these nonlymphoid organs contained a massive population of the B cell phenotype (B220+CD20+) and some T cells (CD3+) (Figure 2D). In addition, Ki67 signal in liver and lung tissue was robust in PELI1-Tg mice, but not in non-Tg mice (Figure 2D). Taken together, these observations indicate that tumors developing in PELI1-Tg mice are likely associated with B cell activation and infiltration.

BM transplantation of hematopoietic stem cells expressing PELI1 leads to development of lymphoid tumors with B cell infiltration. Because PELI1 overexpression contributed to immunological defects and B cell–positive tumor development in PELI1-Tg mice, we next sought to determine whether this tumor development occurs autonomously in the hematopoietic system. BM transplantation (BMT) was performed using hematopoietic stem cells (HSCs) infected with retroviruses encoding human PELI1 plus GFP (Figure 3A). Significant populations of transduced HSCs were maintained in the peripheral blood, LNs, and BM for approximate-
lower in GFP-PELI1 recipient LNs (Figure 3, F and G). In addition, immunohistochemical (IHC) analysis revealed that the lymphoid tissue of GFP-PELI1 recipient mice showed a marked increase in B220+ lymphocytes compared with GFP recipient and Eμ-Myc-Tg mice (Figure 3H). Together, these results demonstrate that PELI1 expression triggered B220+ lymphocyte infiltration, which was likely associated with lymphomagenesis and hematopoietic malignancy in these mice. However, GFP-PELI1 recipient mice developed apparent signs of some lymphoblastic (and potentially leukemic) disorder (e.g., rear-limb paresis) at >40 weeks after transduction, which indicates that PELI1-induced hematopoietic malignancies had a long latency period or were dose dependent. Taken together, these results suggest that PELI1 overexpression leads to malignant expansion of B cells and has some tumorigenic potential in vitro and in vivo.

PELI1 interacts with and promotes BCL6 induction by K63-mediated polyubiquitination. In our initial approach to examining the molecular mechanism underlying PELI1 gain of func-

Figure 1. Development of tumors in mice expressing PELI1 protein. (A) Kaplan-Meier curves of OS for PELI1-Tg mice (n = 23) and non-Tg littermates (n = 20) from 3 independent founder lines (PELI1-Tg lines 2, 3, and 9; non-Tg lines 1, 5, and 6). (B) Macroscopic images of non-Tg littermates (founder line 1) and PELI1-Tg mice (founder line 9). Arrows denote tumors. (C) Total tumor incidence for the indicated PELI1-Tg and littermate non-Tg founder lines, as determined by macroscopic analysis. (D) Representative micrographs of H&E-stained tissue samples from the thymus, spleen, cervical LN, Peyer’s patch, liver, and lungs of non-Tg and PELI1-Tg mice. Original magnification, ×400.
B cell lymphomagenesis using splenic B220+ cells isolated from 

section, we determined the expression profile of a series of repre-

Supprisingly, there was a major difference between control and

\( \text{PELI1} \)

Overexpression of \( \text{PELI1} \) with increasing amounts of \( \text{HA-Ub K63} \) were transf-

To further verify the K63-mediated ubiquitination of BCL6 by

<table>
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<tr>
<th>Tumor site</th>
<th>( \text{PELI1-Tg} )</th>
<th>Non-Tg</th>
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<tr>
<td>LN</td>
<td>10 (20%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Peyer’s patch</td>
<td>6 (12%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Spleen</td>
<td>9 (18%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Thymus</td>
<td>8 (16%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Intestine</td>
<td>7 (14%)</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Liver</td>
<td>19 (39%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Lung</td>
<td>8 (16%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>7 (14%)</td>
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<td>Prostate</td>
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Results are shown for \( \text{PELI1-Tg} \) founder line 9 \((n = 49)\) and non-Tg founder line 1 \((n = 38)\). *Axillary, cervical, inguinal, and mesenteric. *Adenomas.
Figure 2. Constitutive PEL1 expression promotes hematopoietic alterations and activates ligand-independent B cell signal transduction. (A) Proportion of B220+ B cell and CD3+ T cell populations, measured by flow cytometry, in cells isolated from spleen, LN, and thymus of non-Tg and PEL1-Tg mice at 14-16 months of age. Data (mean ± SEM) are representative of 3 independent experiments, with 4 mice per experiment. ***P < 0.001. (B) Flow cytometry of CD86 and MHC class II surface expression in splenic cells derived from non-Tg and PEL1-Tg mice incubated in vitro for 24 hours in the presence of anti-CD40 and anti-IgM antibodies. Gray histograms represent untreated non-Tg cells (unstimulated control). (C) Representative images of H&E staining and IHC analyses for B220, CD3, CD20, and CD23 antigen expression in splenic and thymic tissue samples isolated from non-Tg and PEL1-Tg mice. Strong B220 and CD20 staining was observed in the spleen and thymus of PEL1-Tg mice, whereas B220 and CD20 staining was detectable only at the GC of control non-Tg mice. Original magnification, ×200. (D) Liver and lung tissue samples isolated from non-Tg littermates and PEL1-Tg mice were fixed and stained with anti-B220, anti-CD3, anti-CD20, anti-CD23, and anti-Ki67 antibodies in serial sections. Original magnification, ×200.
Figure 3. BMT of HSCs expressing PELI1 results in development of lymphoid tumors with B cell infiltration. (A) BMT protocol. HSCs derived from primary murine BM were retrovirally transduced with GFP or GFP-PELI1, subjected to 3 consecutive rounds of transduction, and transplanted into sublethally irradiated recipient mice (see Supplemental Methods for details). (B) Flow cytometric quantification of the GFP⁺ cell population in blood, LN, and BM of GFP or GFP-PELI1 recipient mice 25–30 weeks after BMT. (C and D) Representative macroscopic images showing thymic tumors and enlarged LNs of GFP-PELI1 recipient mice. Eμ-Myc-Tg mice were used as a positive control for lymphomagenesis. Ax, axillary; In, inguinal. (E) Representative flow cytometric analysis results for B220⁺ and CD3⁺ cell populations in the thymus of GFP and GFP-PELI1 recipient mice as well as Eμ-Myc-Tg mice. (F) Representative flow cytometric analysis for B220⁺ and CD3⁺ cell populations in LNs of GFP and GFP-PELI1 recipient mice. Ce, cervical. (G) Flow cytometric quantification of B220⁺ and CD3⁺ cell populations in LNs of control GFP (n = 4) and GFP-PELI1 (n = 5) recipient mice at 30 weeks after BMT (data represent mean ± SEM). *P < 0.05, **P < 0.01. (H) Representative images of IHC analyses for B220 antigen expression in LN and thymic tissue samples isolated from GFP and GFP-PELI1 recipient mice as well as Eμ-Myc-Tg mice. Original magnification, ×200; Scale bars: 200 μm.
B cell development in PELI1-Tg mice. B cell development in the BM and spleen was analyzed using flow cytometry. There was no obvious difference in B220+ and CD3+ lymphocyte subpopulations in the BM and spleen between young PELI1-Tg and non-Tg mice (Supplemental Figure 6). However, old PELI1-Tg mice exhibited significant increases (average 2.4-fold increase compared with non-Tg) in B220+IgMloIgD+ and decreases in pre-B cell (B220+IgM–IgD–) and/or immature B cell (B220+IgM+IgD–) populations (Figure 6A). Similarly, the population of CD43-gated mature B cells (B220hiCD43–IgM+) showed a substantial increase in PELI1-Tg versus non-Tg mice, whereas pre-B cell (B220+CD43 IgM+) and immature B cell (B220+CD43 IgM+) populations decreased (Figure 6B). These findings indicate that B cell–related tumor development in PELI1-overexpressing mice is related mainly to an increase in mature B cells.

In lymphoid tissue, expression of the membrane metalloproteinase CD10 was largely restricted to GCB cells, but that of MUM1/IRF4 was limited to post-GCB cells (used herein to refer to both post-GCB cells and/or ABC-like malignancies) (11). Many studies have suggested a positive correlation between CD10 and BCL6 expression and a negative correlation between CD10 and MUM1/IRF4 expression (21). However, expression of BCL6...
Figure 5. **PELI1 expression promotes BCL6 stabilization.** (A) Cellular extracts from 9 B cell lymphoma cell lines were immunoblotted with the indicated antibodies. (B) DOHH2 cells were transfected with control luciferase shRNA (shLuc), PEL1 shRNA (shPELI1; targeting PELI1 ORF), or 3′ untranslated region PELI1 shRNA (3′ UTR; targeting PELI1 3′ UTR) encoding construct in combination with the GFP or GFP-PELI1 expression plasmid, and SU-DHL4 cells were transfected with the pMyc or pMyc-PELI1 expression plasmid in combination with the shLuc or 3′ UTR encoding construct. (C) HeLa cells were transfected with control pMyc, pMyc-PELI1-FL, or pMyc-PELI1-ΔC and HA-BCL6. At 24 hours after transfection, cells were cultured in the presence of LPS, further treated with cycloheximide (CHX), and lysed at the indicated times. (D) Splenic B220+ cells were isolated from non-Tg and PELI1-Tg mice and maintained in the absence or presence of LPS. At 24 hours after treatment, splenic B220+ cells were lysed and subjected to immunoblotting. In (B–D), expression levels relative to the respective control are shown below blots. (E) B220+ cells were isolated from BM, LNs, and spleen of non-Tg and PELI1-Tg mouse–derived B220+ cells. Data (mean ± SEM) are representative of 2 experiments with 3 mice per experiment. ***P < 0.001.
Correlation between PELI1 and BCL6 expression in human DLBCLs. To further examine the relationship between PELI1 expression and BCL6 expression in human cancer, PELI1 and BCL6 levels were assessed in 113 patients with DLBCLs classified according to World Health Organization guidelines (Figure 7). DLBCLs represent the most common form of non-Hodgkin lymphomas (NHLs) worldwide, accounting for approximately 40% of all new cases of lymphomas and 68% of all NHL cases among adult patients in Korea (13, 22). In addition, BCL6 is known to facilitate lymphomagenesis of DLBCLs (12). The intensity of PELI1 expression was highly correlated with BCL6 expression, particularly in these 4 cases of DLBCL patients. Specifically, DLBCL patients with intense cytoplasmic and nuclear PELI1 expression showed high BCL6 expression (Figure 7A). In addition, DLBCL patients with moderate and weak expression of cytoplasmic and/or nuclear PELI1 protein showed parallel moderate and weak induction, respectively, of BCL6 protein. These results are indicative of an important positive correlation between PELI1 and BCL6 expression levels in DLBCLs.
translocation–negative DLBCL patients showed some mutation at G170, G229, and C236 bases of the 5′-noncoding region of BCL6 (Supplemental Figure 8). However, it remains unclear whether these mutations affect BCL6 transcripational activity.

IHC staining for PELI1 and BCL6 was scored according to staining intensity (scores ranging 0–3) and the extent of positive
The chronic or constitutive activation of signaling mediated by the BCR is a key process in lymphomagenesis induced by the activation of cell survival signals (26, 27). Therefore, the ability to induce this signaling is compatible with the survival of B cells and the development of a B cell tumor. The constitutive stimulation of BCR, CD40, and BAFF receptors and various TLRs activates the NF-κB pathway in B cells. Activation of NF-κB induces the expression of IRF4 and subsequently that of the master regulator of plasmacytic survival (Supplemental Figure 9). These observations are indicative of a relationship between PELI1 expression and poor survival rate in DLBCL patients, implying the prognostic value of PELI1 expression. According to a multivariate Cox regression analysis integrating various risk factors, including age, sex, stages, B symptoms, bulky diseases, BM involvement, BCL6 translocation, EBV in situ hybridization, number of extranodal sites, and PELI1 expression, PELI1hi status was an independent prognostic predictor of low OS (HR, 3.745;  \( P = 0.001 \); Supplemental Table 4). In addition, PELI1hi was an independent prognostic indicator of international prognostic index score (hazard ratio, 2.443;  \( P = 0.010 \)). Together, these results indicate that PELI1 expression serves as a novel oncogenic signal and that PELI1 facilitates lymphomagenesis through BCL6 stabilization.

Discussion

Many B cell lymphomas begin at the GC of B cells and by deregulation of BCL6 expression, which is induced by some chromosomal rearrangement (14, 15) and point mutation of the BCL6 promoter region (16). However, deregulation of BCL6 expression is not based solely on these genetic alterations. Recently, BCL6 has been found to be degraded by an SCF Ub ligase complex containing FBXO11, which is inactivated in DLBCLs (17). In the present study, PELI1 was found to upregulate oncogenic BCL6 by K63-linked polyubiquitination, and its expression constitutively activated ligand-independent B cell signal transduction and developed a variety of lymphoid tumors with prominent B cell infiltration. These results suggest that PELI1 expression may confer increased proliferative and tumorigenic advantages to B cells by activating BCL6 (Figure 8).

PELI1 is a signal-responsive E3 Ub ligase that promotes the K48-linked polyubiquitination of c-Rel for ATP-dependent proteolysis by the proteasome (23), whereas K63-linked Ub chains mediate nonclassical, degradation-independent modifications (24), such as TRAF-6 activation (25). Recent studies suggested that the stimulation of BCR signaling in response to TLR3 and TLR4 agonists activates the expression and E3 ligase activity of PELI1 (6, 8, 9). Notably, the ligand-independent activation of receptor-mediated signal transduction plays an active role in the tumorigenic process in humans. In our current study, constitutive expression of PELI1 induced the ligand-independent hyperactivation of B cell signal transduction. In turn, PELI1 induced BCL6 upregulation and activated the proliferation and survival signaling of B cells. These findings suggest that PELI1 expression may be an important mechanism underlying oncogenic BCL6 overexpression in human B cell lymphomas. PELI2 and PELI3 are known to serve as E3 Ub ligases that catalyze K63-linked polyubiquitination (25); thus, it is likely that all PELI family proteins interact with and promote BCL6 induction by K63-mediated polyubiquitination.

The oncogenic potential of PELI1 is supported by the observation that PELI1 protein expression is also associated with high-level BCL6 expression (Spearman \( r = 0.517;  \( P < 0.001 \)). These results demonstrate a significant positive correlation between PELI1 and BCL6 levels in DBCLs. However, with DBCL cases classified into GCB and non-GCB types using the Hans algorithm, PELI1 expression showed a positive correlation with BCL6 in both groups (Supplemental Tables 2 and 3).

PELI1 overexpression closely associates with poor prognosis in DLBCL patients. We next examined the prognostic implications of PELI1 expression in DLBCLs. DLBCL patients were assigned to 2 groups based on their PELI1 expression. A receiver operating characteristic (ROC) curve analysis was conducted to determine the optimal cutoff point for 10% positive cells for PELI1 staining indices. Here, a patient was deemed PELI1hi if at least 10% of tumor cells expressed PELI1 with moderate to strong intensity and nuclear expression (Figure 7A and Supplemental Table 2); all other patients were PELI1lo. OS was compared between PELI1lo and PELI1hi groups (Figure 7E).

Notably, OS reached almost 68%–70% in the PELI1lo group (at approximately 36 months;  \( P = 0.045 \)). OS in the PELI1hi group gradually decreased over the long-term 120-month follow-up period (almost 42% decrease at approximately 36 months;  \( P = 0.003 \); Figure 7E).

Among the 71 patients with R-CHOP treatment, PELI1hi status was also significantly associated with decreased OS ( \( P = 0.045 \)), similar to that of all 113 DLBCL patients in Kaplan-Meier survival analysis (Supplemental Figure 9). These observations are indicative of a relationship between PELI1 expression and poor survival rate in DLBCL patients, implying the prognostic value of PELI1 expression. According to a multivariate Cox regression analysis integrating various risk factors, including age, sex, stages, B symptoms, bulky diseases, BM involvement, BCL6 translocation, EBV in situ hybridization, number of extranodal sites, and PELI1 expression, PELI1hi status was an independent prognostic predictor of low OS (HR, 3.745;  \( P = 0.001 \); Supplemental Table 4). In addition, PELI1hi was an independent prognostic indicator of international prognostic index score (hazard ratio, 2.443;  \( P = 0.010 \)). Together, these results indicate that PELI1 expression serves as a novel oncogenic signal and that PELI1 facilitates lymphomagenesis through BCL6 stabilization.
PELI1 expression is attenuated in normal B and T cells, but is significantly attenuated by PELI1 deficiency (2). Lack of NF-κB is associated with immune deficiencies, and abnormal NF-κB activation is associated with autoimmune diseases and cancer. In particular, activation of NF-κB signaling is susceptible to the avoidance of apoptotic cell death in the pathogenesis of certain B cell lymphomas, such as the ABC subtype of B cell lymphomas, Hodgkin’s lymphoma, primary mediastinal B cell lymphomas, lymphoid tissue lymphomas associated with the gastric mucosa, and multiple myeloma (11). However, aberrant activation of NF-κB signaling occurs in fewer than 10% of cases involving the GCB subtype (11), which indicates that constitutive activation of NF-κB signaling is not a common pathway by which GC B signaling occurs in fewer than 10% of cases. Instead, PELI1 expression is attenuated in normal B and T cells, but is significantly attenuated by PELI1 deficiency (2). Lack of NF-κB is associated with immune deficiencies, and abnormal NF-κB activation is associated with autoimmune diseases and cancer. In particular, activation of NF-κB signaling is susceptible to the avoidance of apoptotic cell death in the pathogenesis of certain B cell lymphomas, such as the ABC subtype of B cell lymphomas, Hodgkin’s lymphoma, primary mediastinal B cell lymphomas, lymphoid tissue lymphomas associated with the gastric mucosa, and multiple myeloma (11). However, aberrant activation of NF-κB signaling occurs in fewer than 10% of cases involving the GCB subtype (11), which indicates that constitutive activation of NF-κB signaling is not a common pathway by which GC B cell lymphomas avoid cell death. In the present study, mice constitutively expressing PELI1 displayed significant downregulation of upstream inhibitory (e.g., plkB and IKKζ) and inactive precursor (e.g., NF-κB1 p105) molecules involved in classical NF-κB signaling (Supplemental Figure 4). However, we did not observe induction of active forms of NF-κB signaling, such as p65, in B cells of PELI1-Tg mice. This suggests that the lymphogenic survival signaling induced by constitutive PELI1 expression may be independent of the NF-κB pathway.

PELI1 expression is attenuated in normal B and T cells, but is activated in response to signaling mediated by BCRs, TCRs, and TLRs (2, 5, 6). In the current study, PELI1 expression was attenuated in various cell lines, except for some hematopoietic and immune cells, which is consistent with previous research (6) and suggests that PELI1 overexpression may occur in a cell type- or tissue-specific manner. The acquisition of genetic mutations that directly affect PELI1 transcription may not be the only mechanism


