Class IA Phosphatidylinositol 3-Kinase Inhibition Inhibits Cell Growth and Proliferation in Mantle Cell Lymphoma

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Key Words

Akt · BCR signaling · Class IA phosphatidylinositol 3-kinase · Mantle cell lymphoma

Abstract

Background/Aims: Constitutive activation of the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin signaling pathway preferentially occurs in aggressive blastoid variants of mantle cell lymphoma (MCL) and is implicated in the pathogenesis of this disease. In this study, we investigated the role of PI3K isoforms on proliferation of aggressive MCL cells. **Methods:** The changes in cell viability, cell cycle distribution and apoptosis induction by the PI3K isoform-selective inhibitors were evaluated. The molecular basis underlying the effects of the specific inhibition of PI3K isoforms was investigated by Western blot analysis. **Results:** Our results demonstrated that a class IA PI3K isoform is most commonly involved in the constitutive activation of Akt in aggressive MCL. Treatment with a p110 α isoform-specific inhibitor induced prominent cell cycle arrest followed by apoptosis through complete abolishment of phosphorylated (p)-Akt and its downstream targets. An inhibitor of isoform p110 δ induced moderate cell cycle arrest with down-

regulation of p-Akt and p-S6K. A dual inhibitor of p110 α and p110 δ GDC-0941 caused more prominent cell growth inhibition compared to selective p110 α or p110 δ inhibitors. Inhibition of the class IB PI3K isoform p110 γ did not cause cell cycle arrest or induce apoptosis in MCL cells. **Conclusion:** These findings suggest that the therapeutic ablation of class IA PI3K may be a promising strategy for the treatment of refractory, aggressive MCL.

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Introduction

Mantle cell lymphoma (MCL) is a subtype of B-cell non-Hodgkin lymphoma characterized by aggressive clinical behavior and, frequently, resistance to chemotherapeutic agents [1]. More than 95% of MCLs show the t(11;14)(q13;32) translocation, which leads to overexpression of cyclin D1 [2]. Although deregulation of the cyclin D1/retinoblastoma protein (Rb) pathway results in instability of the G₁/S checkpoint [2], overexpression of cyclin D1 alone is not sufficient for the development of MCL, and additional genetic events appear to be necessary for oncogenesis [3].

It has been demonstrated that constitutive activation of the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway plays a critical role in the pathogenesis of aggressive blastoid variants of MCL [4, 5]. Promising results from several phase II and III trials of the PI3K/Akt/mTOR inhibitor temsirolimus (CCI-779) in relapsed or refractory MCL provide further clinical evidence of the importance of this pathway in MCL [6]. PI3K is the major activator of Akt, a serine/threonine protein kinase that modulates a variety of downstream substrates involved in the regulation of cell cycle progression, cell survival, and transcription. PI3Ks are divided into three classes, class I, II, and III, and only the class I PI3Ks are able to generate the second messenger phosphatidylinositol 3,4,5-trisphosphate through activation of phosphoinositide-dependent kinase-1, which subsequently phosphorylates and activates Akt [7]. Class I PI3Ks are further classified into subclasses IA and IB. Class IA PI3Ks, namely PI3K α , PI3K β , and PI3K δ , are composed of catalytic subunits p110 α , p110 β , and p110 δ , respectively, which bind to a set of alternative regulatory subunits (including p85 α , p85 β , p55 α , p50 α , and p55 γ) and are activated by receptor tyrosine kinases and Ras [8]. In contrast to p110 α and p110 β , which have wide tissue distribution, p110 δ is expressed mainly in leukocytes [9].

PI3K δ has a dominant role in B-cell responses initiated by antigen-mediated clustering of the B-cell receptor (BCR) via selective recruitment of p110 δ and sequential activation of its downstream pathways [10, 11]. However, in ligand-independent 'tonic signaling', p110 α seems to perform redundant functions for signaling activation, and both PI3K α and PI3K δ isoforms have been implicated as mediators of agonist-independent constitutive BCR/PI3K/Akt signaling [11]. We have demonstrated that constitutive activation of the PI3K/Akt pathway occurs preferentially in aggressive blastoid variants but not in typical MCL [5]. In addition to its role in BCR signaling, the p110 α isoform plays a particularly important role in tumorigenesis through both gene amplification and gain-of-function mutations [12]. The class IA PI3K isoform p110 β is known to contribute to tumorigenesis, associating with phosphatidylinositol 3,4,5-trisphosphate production induced by deficiency in PTEN [13], a catalytic antagonist of PI3K [7]. The class IB PI3K γ , with its catalytic subunit p110 γ and regulatory subunit p101 or p84/p87, is activated by G-protein-coupled receptors without affecting BCR signaling [14]. PI3K γ plays an important role in the immune system and is involved in inflammation [15].

The pan-PI3K inhibitors such as LY294002, which inhibit all PI3K classes [16], have not been successful in clinical development, mainly because of their toxic effects and poor pharmacokinetic properties [17]. In aggressive refractory MCL, in which p110 α and p110 δ are most likely to be involved in BCR signaling [11], the efficacy of these inhibitors has not been well defined.

In this study, we investigated the role of class IA PI3K α and PI3K δ isoforms on proliferation of aggressive MCL cells via constitutive activation of the PI3K/Akt pathway utilizing PI3K isoform-specific inhibitors. Our results indicate that both p110 α and p110 δ are involved in ligand-independent PI3K/Akt activation.

Materials and Methods

Reagents

The pan-PI3K inhibitor LY294002, p110 α -selective inhibitor designated PI3K α inhibitor IV {3-[4-morpholinothieno(3,2-D)pyrimidin-2-yl]phenol dihydrochloride}, and p110 β -selective inhibitor TGX-221 were purchased from Calbiochem (San Diego, Calif., USA). The selective inhibitor of p110 γ AS-605240 was obtained from Sigma-Aldrich (St. Louis, Mo., USA), and the p110 δ -selective inhibitor IC87114 was from Symansis (Auckland, New Zealand). The class I PI3K-specific inhibitor GDC-0941 was obtained from Genentech Inc. (San Francisco, Calif., USA).

Cell Lines and Culture Conditions

Three MCL cell lines, Granta 519 [18], JVM-2 [19], and Jeko-1 [20], were used in this study. Granta 519 and JVM-2 express wild-type (wt) p53, while Jeko-1 is mutant for p53 (loss of p53 expression) [20]. JVM-2 and Jeko-1 cells were cultured in RPMI 1640 medium containing 15% fetal bovine serum (FBS) and 1% penicillin/streptomycin, while Granta 519 was grown in Dulbecco's modified Eagle's medium supplemented with 15% FBS and 1% penicillin/streptomycin. Primary MCL samples were obtained after informed consent in accordance with institutional guidelines set forth by Aichi Medical University and Saga University per Declaration of Helsinki principles. Mononuclear cells were purified by Ficoll-Hypaque (Sigma-Aldrich) density gradient centrifugation, and nonadherent cell were resuspended in RPMI 1640 medium supplemented with 10% FBS at a density of 6×10^6 cells/ml. Clinical characteristics of the patients are summarized in online supplementary table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000353164).

For cell viability assays, Western blot, and cell cycle analysis, cells were first acclimated in RPMI 1640 or Dulbecco's modified Eagle's medium containing 5% FBS for 16 h prior to exposure to inhibitors. Control cells were treated with an equivalent amount of DMSO under the same growth conditions.

Cell Viability/Proliferation Assay

Cell viability was assessed by the trypan blue dye exclusion method, and cell proliferation was determined by the CellTiter 96 AQueous One Solution Cell Proliferation Assay (MTS; Promega,

Madison, Wisc., USA) according to the company's protocol. Optical density was measured using a SpectraMax 340PC (Molecular Devices, Sunnyvale, Calif., USA).

Apoptosis and Cell Cycle Analysis

Apoptotic cell death was analyzed by annexin V staining with fluorescein isothiocyanate-conjugated annexin V (Roche Diagnostics, Indianapolis, Ind., USA) and propidium iodide (PI) [21]. The extent of drug-specific apoptosis was assessed by the formula: percent specific apoptosis = (test – control) × 100/(100 – control). Cell cycle distribution was analyzed by flow-cytometric analysis of PI-stained nuclei [21]. Annexin V fluorescence or PI-stained DNA content was determined by a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif., USA). Flow-cytometric data were analyzed by CellQuest software (Becton Dickinson). Gating was set to exclude cell debris, cell doublets, and cell clumps.

Western Blot Analysis

Total cell lysates were collected as described elsewhere [5]. Total protein (30 µg) was separated by SDS polyacrylamide gel electrophoresis (Bio-Rad Laboratories, Hercules, Calif., USA), transferred to polyvinylidene fluoride membranes, and then probed with primary and secondary antibodies according to the manufacturers' protocols. For immunoblotting, the following antibodies were used: α-tubulin (Sigma-Aldrich), p110δ (EMD Millipore, Billerica, Mass., USA), p110α, p110β, p110γ, phosphorylated (p)-Akt^{Ser473}, p-Akt^{Thr308}, p-Rb^{Ser780}, cyclin D1, p-GSK3β^{Ser9}, p-S6K^{Ser240/244}, p-4E-BP1^{Thr37/46}, p-mTOR^{Ser2448}, and horseradish peroxidase-linked anti-mouse and anti-rabbit IgG (all from Cell Signaling Technology, Beverly, Mass., USA).

Small Interfering RNA Transfection

PI3K p110δ gene expression was silenced by small interfering RNA (siRNA) using Stealth RNAi (Invitrogen, Carlsbad, Calif., USA). The sense strand of the siRNA silencing (p110δ-siRNA) was UCUUAAAGAUGAUGCCACGCUGCC (NM_005026.3, 2522–2546), UCAUGAUGUUGUCGCGUGGCCGAUC (NM_005026.3, 2885–2909), and UACUGGAUGUCUUUGGAG-CAGCUGA (NM_005026.3, 3174–3198).

As a nonspecific control, a Stealth RNAi siRNA Negative Control Med GC (Invitrogen) was used. Transfection was facilitated by the Lipofectamine RNAiMAX transfection reagent (Invitrogen) following the manufacturer's instructions. Gene silencing was verified by Western blot performed 48 h after transfection.

Results

PI3K Isoforms Are Expressed in MCL Cells

To establish the baseline status of the pathway, we first assessed the expression levels of PI3K isoforms in the MCL cell lines Jeko-1, JVM-2, and Granta 519. Western blot analysis revealed that the tested cells expressed p110α, p110β, and p110δ (fig. 1), which was consistent with a previous report that leukocytes usually contain all three class IA PI3K subclasses [9]. The expression level of p110α was higher in Jeko-1 and JVM-2 than in Granta 519. The

Fig. 1. Expression levels of PI3K isoforms in MCL cells. Expression levels of PI3K isoforms were assessed in untreated MCL cells by Western blot. Representative results from 3 independent experiments are shown.

tested MCL cell lines demonstrated similar intermediate expression levels of p110δ. The MCL cells also expressed class IB p110γ.

Inhibition of PI3Kα Induces Cell Cycle Arrest in MCL Cells

We next assessed the effects of various PI3K inhibitors on MCL cell cycle progression and apoptosis. As shown in figure 2, treatment with PI3Kα inhibitor IV or nonspecific PI3K inhibitor LY294002 impeded cell cycle progression, with accumulation of cells in the G₀/G₁ phase and depression of the S-phase fraction, in a dose-dependent manner. PI3Kα inhibitor IV (5 µM) increased the G₀/G₁ fraction by a mean of 29.1 ± 4.2% for Jeko-1, 12.2 ± 3.6% for JVM2, and 20.3 ± 3.2% for Granta 519 compared to the same fraction in control cells. LY294002 (5 µM) increased the G₀/G₁ fraction by a mean of 14.8 ± 2.4, 13.3 ± 2.5 and 16.5 ± 3.4% for Jeko-1, JVM2, and Granta 519, respectively, compared to the same fraction in controls. Although recent reports raise the possibility that the p53 status could affect the blocking of PI3K/Akt/mTOR signaling by PI3K inhibitors [5, 22], no significant differences in the sensitivity to specific PI3K isoform inhibitors was observed between wt p53-expressing cells (JVM-2 and Granta 519) and p53-mutant cells (Jeko-1). On the contrary, no increases in the G₀/G₁ fraction were detected in cells treated with IC87114, TGX115, or AS-605240 (fig. 2). While PI3Kα inhibitor IV and LY294002 exhibited dose-depen-

Fig. 2. Effects of PI3K isoform-specific inhibitors on cell cycle in MCL cells. Jeko-1, JVM-2, and Granta 519 cells were treated with 1 or 5 μM of PI3K α inhibitor IV, TGX-221, AS-605240, IC87114, or LY294002 for 48 h and processed for flow-cytometric analysis by PI staining. Graphs represent average percentages of cell populations in each cell cycle phase ($\pm$ SEM) from 3 independent experiments.

dent cell growth-inhibitory activity in the tested cells as assessed by the MTS assay (IC_{50} at 48 h: PI3K α inhibitor IV, 10.7 μM for Jeko-1, 3.2 μM for JVM2, and 9.3 μM for Granta 519; LY294002, 8.1 μM for Jeko-1, 7.4 μM for JVM2, and 9.4 μM for Granta 519), IC87114 induced moderate inhibition of cell growth (fig. 3a). Treatment with PI3K α

inhibitor IV or LY294002 reduced cell viability as demonstrated by concentration-dependent increases in the percentage of trypan blue-positive cells (fig. 3b). We have confirmed induction of apoptotic cell death using annexin V flow cytometry in Jeko-1 cells (fig. 3c).

PI3K α and PI3K δ Inhibit the PI3K/Akt/mTOR Pathway and Downregulate Cell Cycle-Related Proteins

To assess the inhibitory efficacy of PI3K α inhibitor IV on the PI3K/Akt/mTOR pathway, we performed Western blot analysis of p-Akt and mTOR downstream target p-S6K, two markers of PI3K/Akt/mTOR signaling activation [17]. In all tested MCL cell lines, p-Akt and p-S6K were clearly expressed at baseline and fully inhibited following treatment with PI3K α inhibitor IV (fig. 4a). To exclude the possibility that PI3K/Akt signaling was activated by growth factors present in the serum [5], we confirmed the constitutive activation of Akt after 24 and 48 h of conditioning in serum-free medium, and the nonspecific PI3K inhibitor LY294002 also inhibited expression of p-Akt and its downstream targets (data not shown). The p110 δ inhibitor IC87114 downregulated p-Akt and p-S6K in Jeko-1 and Granta 519 cells but to a lesser extent than PI3K α inhibitor IV (fig. 4a). To investigate the molecular basis underlying cell cycle arrest by PI3K α inhibitor IV, we evaluated the expression of several cell cycle-regulatory proteins. The high expression levels of cyclin D1 were downregulated by PI3K α inhibitor IV, and substantial decreases in Rb phosphorylation were also detected. Levels of GSK-3 β kinase, a direct downstream target of Akt that has been shown to negatively regulate cell cycle progression through cyclin D1 repression in MCL cells [4], were decreased by PI3K α inhibitor IV in a concentration-dependent manner. Likewise, levels of 4E-BP1, a downstream target of mTOR known to enhance cyclin D1 translation, were also decreased by PI3K α inhibitor IV. Treatment with IC87114 induced moderate decrease in p-Akt, p-S6K, and p-4E-BP1 levels in both Jeko-1 and Granta 519 cells, while no significant change in p-GSK-3 β , cyclin D1, or p-Rb was observed. The phosphorylation of forkhead transcription factor/forkhead box O3a, another positive regulator of cyclin D1, showed no significant changes in response to PI3K α inhibitor IV or IC87114 (data not shown).

To assure the specificity of the observed cellular effects upon PI3K pharmacological inhibition, we evaluated the consequences of siRNA-mediated silencing of p110 δ in Granta 519 cells. siRNA against p110 δ caused downregulation not only of p110 δ but also its regulatory partner

Fig. 3. Effects of PI3K isoform-specific inhibitors on cell viability in MCL cells. Jeko-1, JVM-2, and Granta 519 cells were treated with the indicated doses of PI3K α inhibitor IV, IC87114, or LY294002 for 48 h. **a** Inhibition of cell proliferation was deter-

mined by MTS assay. **b** Cytotoxic effects were detected by trypan blue staining. Graphs at the top represent FACS data, while the bar graphs represent average percentages $\pm$ SEMs from 3 independent experiments. * $p < 0.05$, ** $p < 0.01$.

p85 along with considerable decreased expression of other PI3K isoforms (p110 α , p110 β , and p110 γ ; online suppl. fig. 1), consistent with published findings in glioma cells [23]. Furthermore, p110 δ siRNA completely abrogated Akt phosphorylation which was only partially diminished by IC87114. These results suggest that genetic disruption of a specific PI3K isoform, unlike pharmacological inhibitors, may affect other important components of PI3K/Akt signaling.

Antitumor Efficacy of the p110 α /p110 δ Dual Inhibitor GDC-0941 in MCL Cells

We next investigated MCL cell responsiveness to the concomitant inhibition of p110 α /p110 δ by the novel

class I PI3K-selective inhibitor GDC-0941 [24]. The potency of GDC-0941 against phosphorylated protein biomarkers of the PI3K pathway p-Akt and its downstream target p-GSK3 β has been confirmed by Western blot analysis (fig. 5a). We next monitored viability of MCL cells treated with GDC-0941. GDC-0941 at submicromolar concentrations caused significant cell growth inhibition, substantiating higher activity compared to PI3K α inhibitor IV or IC87114 (IC₅₀ at 48 h: 0.2 μM for Jeko-1, 0.3 μM for JVM-2, and 0.8 μM for Granta 519; fig. 5b). This was associated with an increase in trypan blue-positive dead cells (fig. 5c) and accumulation of cells in the G₀/G₁ phase with corresponding depletion of proliferating cells with S-phase DNA content (fig. 5d).

Fig. 3. Effects of PI3K isoform-specific inhibitors on cell viability in MCL cells. Jeko-1, JVM-2, and Granta 519 cells were treated with the indicated doses of PI3Kα inhibitor IV, IC87114, or LY294002 for 48 h. **c** Apoptosis induction was measured by flow-

cytometric analysis with annexin V/PI staining. Graphs at the top represent FACS data, while the bar graphs represent average percentages $\pm$ SEMs from 3 independent experiments. * $p < 0.05$, ** $p < 0.01$.

We also utilized cells from two primary MCL patients (online suppl. table 1) to assess the antiproliferative effects of p110α inhibition by PI3Kα inhibitor IV, p110δ inhibition by IC87114, or dual p110α/p110δ inhibition by GDC-0941. GDC-0941 treatment induced more prominent cell growth inhibition and increase in trypan blue-positive dead cells than PI3Kα inhibitor IV or IC87114 in both samples (fig. 6).

Discussion

Our findings show that the growth of MCL cell lines with constitutive activation of PI3K/Akt/mTOR signaling was attenuated by inhibition of the PI3K p110α isoform, and that this growth inhibition was related primar-

ily to G₀/G₁ cell cycle arrest and moderate apoptotic responses. In our system, treatment of MCL cells with the p110δ-specific inhibitor IC87114 resulted in limited growth inhibition, with moderate downregulation of p-Akt, p-S6K, and p-4E-BP1, and almost no change in p-GSK-3β, cyclin D1, or p-Rb levels compared to the more robust inhibition seen with the p110α PI3Kα inhibitor IV. In turn, dual inhibition of p110α and p110δ by GDC-0941 caused more prominent cell growth inhibition compared to the single isoform-targeted inhibitors.

Several reports have implicated the PI3Kα isoform in PI3K/Akt oncogenic activation in B-cell lymphoma/leukemia, including MCL [25, 26]. In MCL cells, a frequent gain of *PIK3CA* gene copy number, but not activating mutations of the *PIK3CA* gene, has been shown to contribute to oncogenic activation of the PI3K/Akt pathway

Fig. 4. Western blot analysis of PI3K/Akt/mTOR signaling and cell cycle-related proteins. Jeko-1 and Granta 519 cells were treated with the indicated dose of PI3Kα inhibitor IV or IC87114 for 24 h, subjected to lysis, and analyzed by Western blot. Changes in the expression levels of p-Akt^{Ser473}, p-S6K^{Ser240/244}, cyclin D1, p-Rb^{Ser780}, p-GSK3β^{Ser9} and p-4E-BP1^{Thr37/46} are shown. Intensity of the immunoblot signals after background subtraction was quantified using ImageJ software, and the relative intensity compared with that of α-tubulin was calculated. Representative results from 3 independent experiments are shown.

[26]. Whereas p110α and p110δ inhibitors have reproducible inhibitory effects on the viability of chronic lymphoid leukemia (CLL) cells and on Akt phosphorylation, the p110α effects were notably stronger than the p110δ effects [25]. On the other hand, the p110δ isoform is known to play a critical role in BCR signaling [27] and is indispensable for BCR-induced DNA synthesis and activation of PI3K/Akt/mTOR signaling [28]. A previous study reported that the p110δ inhibitor CAL-101, an orally active derivative of IC87114, promoted apoptosis in primary CLL cells in which Akt phosphorylation is dependent on CD40 ligation and stromal contact [29]. These findings suggest that the prosurvival effects of PI3K activation in B-cell malignancies are mediated through the combined activation of p110α and p110δ, with higher dependency upon p110α [25].

Lannutti et al. [30] reported that the p110δ inhibitor CAL-101 blocked Akt^{Ser473} phosphorylation in patient-derived MCL cells following BCR crosslinking and in Jeko-1 cells upon activation of CXCR5, CXCR4, BCR, or BAFF (B-cell-activating factor belonging to the TNF family), but these investigators did not report the effects on cell growth. In our study, though we did observe mild inhibition of growth of all tested MCL cells following treatment with IC87114, this PI3Kδ inhibitor was not

nearly as effective in inhibiting cell growth as was the PI3Kα inhibitor IV. At difference with small-molecule inhibitors, silencing of p110β by siRNA completely diminished Akt phosphorylation, but this was likely caused by concomitant alterations in its regulatory part-

Fig. 5. Effects of the p110α and p110δ dual inhibitor GDC-0941 in MCL cells. **a** Jeko-1, JVM-2, and Granta 519 cells were treated with GDC-0941 (1 μM) for 24 h, subjected to lysis, and analyzed by Western blot. Changes in the expression levels of p-Akt^{Ser473} and p-GSK3β^{Ser9} are shown. Intensity of the immunoblot signals after background subtraction was quantified using ImageJ software, and the relative intensity compared with that of α-tubulin was calculated. Representative results from 3 independent experiments are shown.

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Fig. 5. Effects of the p110 α and p110 δ dual inhibitor GDC-0941 in MCL cells. **b–d** Jeko-1, JVM2, and Granta 519 cells were treated with the indicated doses of GDC-0941 for 48 h. After treatment, inhibition of cell proliferation was determined by MTS assay (**b**), cytotoxic effects were detected trypan blue staining (**c**), and induction of cell cycle arrest was measured by flow-cytometric analysis by PI staining (**d**). Graphs represent average percentages $\pm$ SEMs from 3 independent experiments. * $p < 0.05$, ** $p < 0.01$.

Fig. 6. Effects of PI3K isoform-specific inhibitors in primary MCL cells. Primary MCL cells were incubated for 48 h with PI3K α inhibitor IV, IC87114, or GDC-0941. Clinical characteristics of patients 1 (**a**) and 2 (**b**) are summarized in the online supplementary table 1. Viable cell number was measured by trypan blue exclusion.

ner p85 and decreased expression of other PI3K isoforms, highlighting previously reported discrepancies between RNA interference and small-molecule PI3K isoform inhibitors [23, 31]. Notably, Lannutti et al. [30] observed no constitutive activation of Akt at the phosphorylation residue of Ser⁴⁷³ in MCL cells that they studied, whereas we found constitutive Akt^{Ser473} phosphorylation in MCL cell lines and in a subset of MCL cases [5]. It is known that B cells utilize both p110 α and p110 δ and that these have overlapping functions at various stages of development [11]. It is possible that agonist-independent constitutive BCR/PI3K/Akt signaling activation in aggressive MCL cells and cell lines [32] is less dependent on p110 δ than the signaling occurring following BCR ligand or cytokine activation in CLL or classic MCL. Our study differed from that of Lannutti et al. [30] by focusing on activation of agonist-independent constitutive BCR/PI3K/Akt signaling without BCR crosslinking or chemokine/cytokine activation. Granta 519 has amplification of the *PIK3CA* gene [25] and Jeko-1 has amplification of the *SYK* gene upstream of PI3K [33]. The amplification or constitutive activation through an upstream activator might be a reason for the increased sensitivity to the p110 α inhibitors. Herman et al. [29] further demonstrated the effects of p110 δ inhibition, which may disrupt the protection of CLL cells by stromal cells through diminishing production of B-cell-activating factors TNF- α , CD40L, and fibronectin by stromal cells. Chronic active BCR signaling has been implicated as a new pathogenetic mechanism, suggesting the possibility of novel therapeutic strategies [10] and that the effects of p110 δ inhibition within the microen-

vironment of MCL cells, which we did not investigate in this study, could be indispensable to such therapeutic strategies.

Recently, novel PI3K isoform-specific inhibitors have been developed as potential chemotherapeutic drugs and have entered clinical trials [17]. Dual inhibition of p110 α and p110 δ by GDC-0941 translated into more pronounced antitumor effects compared to selective p110 α or p110 δ inhibitors in both MCL cell lines and MCL primary samples. These results indicate that simultaneous inhibition of p110 α and p110 δ by small-molecular inhibitors might be a useful strategy to block survival signals in B-cell malignancies that depend on active BCR tonic signaling [11, 34] and on microenvironment-mediated pro-survival signaling.

Several negative regulators of Akt and mTOR pro-survival pathways, including AMPK β 1, TSC2, and PTEN, appear to be novel stress-related transcriptional targets of p53 [22]. Our results suggest, however, that the antiproliferative effects of PI3K inhibition were not strengthened by wt p53 expression. This finding is concordant with a recent report indicating that Akt inhibitors are capable of inducing apoptosis in CLL cells irrespective of the TP53 status [35].

In conclusion, our results provide evidence that the specific inhibition of class IA PI3Ks, including p110 α and p110 δ , may contribute to abrogation of the constitutive Akt activation and cell proliferation in aggressive MCL; they also suggest that selective inhibition of these isoforms may be a promising therapeutic approach against the aggressive blastoid variants of MCL.

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