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Emergence of the PI3-kinase pathway as a central modulator of normal and aberrant B cell differentiation

G.V. Baracho[a], **A.V. Miletic**[a], **S.A. Omori**[a], **M.H. Cato**[a], and **R.C. Rickert**[a,*]

[a]Program on Inflammatory Disease Research, Infectious and Inflammatory Disease Center, Sanford-Burnham Medical Research Institute, La Jolla, California 92037, USA

Abstract

Phosphoinositide 3-kinase (PI3K) defines a family of lipid kinases that direct a wide range of cellular processes and cell fate decisions. Since its discovery, and that of its enzymatic antagonist PTEN, much of the focus on PI3K has been on its oncogenic potential. In recent years, studies of PI3K signaling in B lymphocytes have established the importance of this pathway in effecting B cell differentiation and associated molecular events such as V(D)J recombination and class switch recombination. Intriguing new findings also indicate that there is specificity in the PI3K pathway in B cells, including preferential expression or usage of particular PI3K isoforms and counter-regulation by the PTEN and SHIP phosphatases. The role of PI3K adaptor proteins (CD19, BCAP, TC21) has also undergone revision to reflect both shared and unique properties. The emergence of Foxo1 as a critical PI3K regulatory target for B cell differentiation has united membrane proximal regulatory events orchestrated by PI3K/PTEN/SHIP with key transcriptional targets. Insights into the regulation and impact of PI3K signaling has been brought to bear in new treatments for B cell malignancies, and will also be an important topic of consideration for B cell-dependent autoimmune diseases.

Introduction

PI3K is a lipid kinase acting on membrane phosphatidylinositol (PtdIns)(4,5)P_2 to produce PtdIns(3,4,5)P_3. Members of the class IA PI3K subset are heterodimeric molecules consisting of a 110 kDa catalytic subunit (p110α, p110β or p110δ) encoded by individual genes (*Pik3ca, Pik3cb* or *Pik3cd*) and a smaller regulatory subunit (Table I). A single gene (*Pik3r1*) encodes the regulatory isoforms p50α, p55α and p85α, while the *Pik3r2* and *Pik3r3* genes encode p85β and p55γ, respectively (Table I). The regulatory subunits prevent degradation of the catalytic subunit while inhibiting its activity. Binding of the tandem SH2 domains in the regulatory subunit to tyrosine-phosphorylated YXXM motifs releases inhibition of the associated catalytic subunit. p110γ is the sole representative of the class IB enzymes and is activated by G-protein-coupled receptors (GPCR) and regulated by the p84/p101 proteins. Of note, p110α and p110β are ubiquitously expressed, while p110δ and p110γ are expressed primarily in hematopoietic cells (Table I). B cell-specific regulation of the class IA PI3Ks is also conferred by the YXXM-bearing adaptor proteins, which can be

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*CORRESPONDENCE: Program on Inflammatory Disease Research Infectious and Inflammatory Disease Center Sanford-Burnham Medical Research Institute 10901 N. Torrey Pines Rd, 12-2101 La Jolla, California 92037 USA (858) 646-3153 Office (858) 795-5412 robert@sanfordburnham.org.

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transmembrane (e.g. CD19) or cytosolic (e.g. BCAP and TC21) and show receptor-specific activation properties (Fig. 1).

All of the catalytic subunits are capable of generating PtdIns(3,4,5)P_3; however, they also possess unique functions, particularly with respect to crosstalk with the Ras pathway. Ras has been noted to bind and activate p110α,β,γ, but this attribute is not shared by p110δ [1]. Consistently, cells relying on p110δ for oncogenic activity are resistant to inhibitors of the MAP kinase pathway, whereas cells expressing p110α, β and γ lose transforming capacity when treated with MAP kinase inhibitors or when Ras-binding is disabled [2,3]. Moreover, loss of function due to impaired Ras-binding can be compensated for by the provision of a myristylation signal, suggesting a role for Ras in p110α,β,γ recruitment to the membrane [2,3]. Importantly, p110δ is the only isoform that exhibits some level of constitutive activity, and becomes oncogenic upon overexpression [3]. The MAP kinase pathway also intersects at points downstream of PtdIns(3,4,5)P_3 generation, as reviewed elsewhere [4].

Counter-regulation of PI3K activity is achieved by the inositol phosphatases phosphatase and tensin homolog (PTEN) and SH2-containing inositol phosphatase (SHIP) (Fig. 1). PtdIns(3,4,5)P_3 and, perhaps, PtdIns(3,4)P_2 are substrates for the phosphoinositide 3-phosphatase PTEN, which has emerged as the key functional antagonist to PI3K [5]. As such, constitutive PTEN activity counters PI3K activity induced by receptor tyrosine kinases, GPCRs and activated Ras. PTEN functions as a tumor suppressor protein and its loss is likely to activate numerous downstream effector pathways initiated by PtdIns(3,4,5)P_3 binding proteins [6]. In mature B cells, the Ser/Thr kinases Akt and PDK1 and the tyrosine kinase BTK are likely the most critical downstream effectors for PI3K (Fig. 1). All of these molecules possess a pleckstrin homology (PH) domain specific for PtdIns(3,4,5)P_3, allowing for re-localization to the plasma membrane. Interestingly, although many proteins possess PH domains specific for PtdIns(3,4,5)P_3, an additional level of regulation is likely dictated by the relative affinity for PtdIns(3,4,5)P_3 to confer selective recruitment of PH-domain-containing proteins based upon local PtdIns(3,4,5)P_3 abundance. For example, the PH domain of PDK1 has a 20-fold greater affinity for PtdIns(3,4,5)P_3 than the PH domain of Akt [7]. The inositol phosphatase SHIP appears to act primarily by dephosphorylating PtdIns(3,4,5)P_3 at the 5-position and perhaps to some extent on PtdIns(4,5)P_2. Although PTEN and SHIP act on the same primary substrate, it is important to note that they generate distinct lipid products. In fact, a significant fraction of PtdIns(3,4)P_2 is thought to be produced by SHIP-mediated dephosphorylation of PtdIns(3,4,5)P_3 rather than PI3K phosphorylation of PtdIns(4)P. There are fewer known targets of PtdIns(3,4)P_2 than PtdIns(3,4,5)P_3, but they include the adaptor proteins Bam32/DAPP1 and TAPP2.

PI3K signaling in early B cell development

PI3K signaling has been implicated in the differentiation and expansion of pro- and pre-B cells through the sequential dependency on IL-7R and pre-BCR signaling (illustrated in Figure 1). Recent evidence indicates that p110δ and p110α are required in a largely redundant manner for the generation and propagation of pre-B cells, whereas p110β is dispensable [8]. From these and other studies [8,9], it is apparent that the class IB PI3K p110γ does not play an important role in early B cell development. This conclusion contrasts with studies in T cells showing a physical and functional association of p110γ with the pre-TCR and TCR [10,11]. The α subunit of the IL-7R recruits p85α/β to promote PI3K-dependent proliferation [12]. Consistently, in the absence of functional p110α/δ, pro-B cells exhibit impaired IL-7-dependent proliferation [8]. This observation suggests an important autoregulatory loop based upon our recent discovery that the transcription factor Foxo1 drives *Il7ra* expression [13]. In early pro-B cells, PI3K activity is low, allowing Foxo1 to

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remain in the nucleus where it functions to inhibit cell cycle progression by inducing expression of the cell cycle inhibitor $p27^{kip1}$ and repressing cyclin D expression [14-16]. Upon engagement of the IL-7R, PI3K-dependent Akt activation results in the phosphorylation of Foxo1, causing its nuclear exclusion and degradation and allowing cell cycle to proceed. At the same time, Foxo1 inactivation attenuates IL-7R signaling as pre-B cells transit to pre-BCR-dependent growth and proliferation.

In p110α/δ mutant mice, $ckit^{+}CD25^{-}$ pre-B cells accumulate, but do not progress beyond the pre-BCR checkpoint to become $CD25^{+}$ resting pre-B cells [8]. This block was attributed to a failure of pre-B cells to downregulate *Rag* expression, which may be incompatible with genomic stability and allelic exclusion in this rapidly dividing cell population. However, impaired activation of PI3K by the pre-BCR would also promote Foxo1-dependent cellular quiescence. Interestingly, Foxo1 is also required for *Rag* expression [8,17], a process that is negatively regulated by Akt in a BLNK(SLP-65)-dependent manner (Fig. 1) [18]. It is possible that signaling via the SYK/BLNK axis and CD19 have complementary functions, since CD19 promotes Akt activation and the combined loss of BLNK and CD19 results in a complete block in B cell development at the large pre-B stage [19]. A similar block was observed in mice lacking both CD19 and BCAP [20]; the latter of which requires SYK for phosphorylation in chicken DT40 cells [21]. Further resolution of PI3K signaling downstream of the pre-BCR was provided by recent studies of Sin1 and the Rictor-containing mTOR complex (mTORC2) [22]. mTORC2 function is closely aligned with cytoskeleton function, as opposed to the Raptor-containing mTORC1 complex that promotes protein synthesis [23]. Akt activation requires dual phosphorylation by PDK1 at Thr308 and by mTORC2 which is capable of phosphorylating Akt at Ser473 [24]. Consistently, in $Sin1^{-/-}$ B cells, Akt phosphorylation at Ser473 is ablated, but not at Thr308 (Fig. 1) [22]. Specifically, phosphorylation of the Akt2 isoform at Ser473 is required for Foxo1-dependent *Il7ra* and *Rag* expression [22]. Of additional interest, loss of Sin1 does not result in reduced survival, supporting the premise that parsing of signals downstream of PI3K results in distinct cellular outcomes. Thus, while inhibition or downregulation of the pre-BCR allows for Foxo1-mediated *Rag* expression, the pro-proliferation and pro-survival functions of the pre-BCR may also be partially PI3K-dependent. Following successful expression of membrane IgM, subsequent selection of immature B cells is also regulated by the PI3K pathway. PI3K signaling is attenuated via elevated PTEN expression and reduced CD19 signaling in newly formed B cells, conferring increased susceptibility to apoptosis [25,26]. In the absence of PTEN, sustained activation of the PI3K pathway results in a breach of tolerance and the generation of autoantibody-producing cells [26].

PI3K signaling in peripheral B cell function and homeostasis

Attenuated PI3K signaling via the loss of p85α/β, p110δ or adaptor proteins (CD19, BCAP and TC21) results in impaired homeostasis (Fig. 1) [27-33]. Correspondingly, provision of a constitutively active PI3K molecule is sufficient to rescue B cells from apoptosis upon inducible deletion of the BCR [34]. These findings indicate that the PI3K pathway is a primary component of tonic signaling that is required for B cell maintenance. In terms of downstream pathways, recent evidence from the study of $Akt1^{-/-}Akt2^{-/-}$ mice supports a role for Akt in B cell survival [35]. Unexpectedly, however, this function is not via Foxo1 inactivation as Foxo1-deficient follicular B cells exhibit normal survival and show *increased* expression of the general Foxo target and pro-apoptotic factor Bim [13]. Nonetheless, B cell homeostasis is affected by the loss of Foxo1 as it regulates B cell homing to the lymph nodes via *Sell* (CD62L) expression [13]. While studies of B cell homeostasis generally focus on BCR signaling, the BAFF-R also activates PI3K to promote survival as well as priming the cells for growth and division [36,37].

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The PI3K pathway also impacts peripheral B cell differentiation. Studies of impaired BTK function in *xid* mice bearing a mutation in the PH domain of BTK provided early evidence of the importance of the PI3K pathway in peripheral B cells [38,39]. These mice lack B-1 cells and have reduced follicular B cells and responses to TI-2 antigens. The reduction in B-1 and follicular B cells is also observed in $Akt1^{-/-}Akt2^{-/-}$ mice [35]. However, these animals also lack marginal zone B cells, suggesting that Akt-dependent growth and survival are required for this population, while BTK-dependent Ca^{++} responses downstream of the BCR may be dispensable. Correspondingly, loss of PTEN or Foxo1 promotes marginal zone B cell formation and, in the former case, B-1 cell formation [1,40,41]. B-1 and marginal zone B cells are major contributors to antibody responses to multivalent TI-2 antigens. Initiation of these responses is thought to require surface Ig aggregation to confer heightened signaling via transphosphorylation of BCR components and associated molecules, including the adaptor proteins that recruit class IA PI3K heterodimers. By contrast, B cell differentiation induced by protein antigens of low valency require costimulation by T cell-derived factors, leading to the generation of extrafollicular antibody-producing cells or germinal center B cells. Both $CD19^{-/-}$ and $p110\delta^{-/-}$ mice lack germinal centers [28-31], suggesting that recruitment of p110δ to CD19 is required for BCR-dependent selection in the germinal center. Interestingly, however, a recent report assigns the p110δ requirement in the germinal center to T_{FH} cells and not to B cells [42]. Thus, the noted ability of p110α (but not p110β) to bind CD19 may compensate for the loss of p110δ [43]. Moreover, our finding that the PI3K/Foxo1 axis regulates *Aicda* expression supports the view that PI3K signaling may need to be attenuated during germinal center B cell differentiation to allow for AID-dependent *V* gene hypermutation and class switch recombination [13,44]. The importance of the PI3K pathway post-germinal center in the propagation of memory B cells remains to be explored.

The PI3K pathway is of primary interest in cancer where a high degree of oncogenic mutations has been noted [45]. Moreover, inactivation of PTEN is second only to p53 in causal associations with a spectrum of malignancies. Thus, it came as some surprise that PTEN-deficient B cells are not prone to transformation. We recently reported that the absence of B cell tumors in PTEN-deficient mice is due to the coordinate role of SHIP as a tumor suppressor [46]. Hence, B cells lacking PTEN or SHIP do not undergo transformation, whereas the loss of PTEN and SHIP results in an aggressive B lymphoma at high penetrance. Given that SHIP function appears to be restricted to dampening BCR signaling, these findings support the notion that tonic signaling via BCR-dependent PI3K activation is an important determinant of transformation. Consistent with this notion, an inhibitor of BTK has shown efficacy in a spontaneous canine lymphoma model and is currently in phase I clinical trials in patients with B cell malignancies [47]. The unique constitutive activity of p110δ combined with its hematopoietically-restricted expression pattern also makes it an attractive therapeutic target. Indeed, *in vitro* studies have revealed broad efficacy of a p110δ-specific inhibitor in promoting the apoptosis of B cell leukemia and lymphoma lines and is currently under clinical evaluation for the treatment of several B cell malignancies [48]. Some caution may be exerted here, and for other applications of PI3K inhibitors, as it has been documented by us and others that inhibition of PI3K promotes hyper-class switching, which may cause untoward side effects such as hypersensitivity due to aberrant IgE production [44,49,50].

Conclusions

The PI3K pathway has come to the forefront as a critical signaling circuit in B cell differentiation and function. Genetic studies in mice have revealed both common and unique utilization of the PI3K/Akt/Foxo axis. In addition, elucidation of isoform-specific functions of PI3K has provided insight into regulatory aspects of PI3K activity as well as

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opportunities for therapeutic intervention. While our understanding of PI3K signaling downstream of the BCR is fairly advanced, a challenge for the future is to understand the degree to which other receptor systems utilize this pathway and how these multiple signals are integrated in the physiologic setting.

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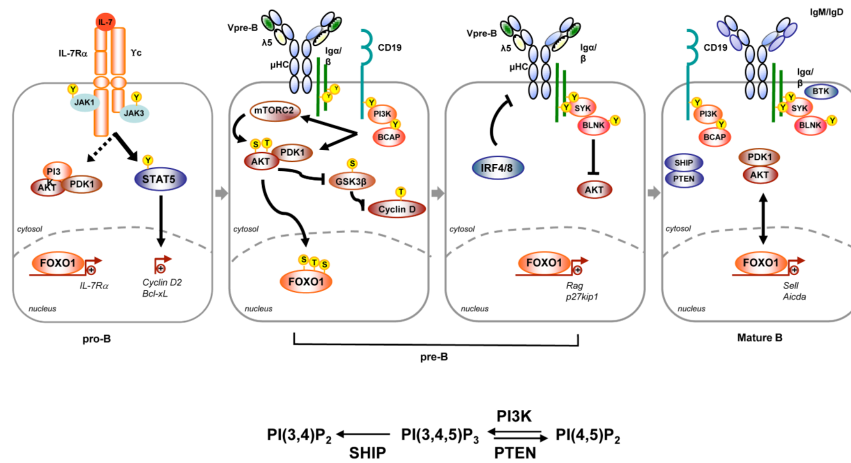

Figure 1. PI3K signaling pathway in B cell development

Pro-B cell development is supported by IL-7 stimulation via the PI3K and STAT5 pathways. Weak PI3K signaling promotes retention of Foxo1 in the nucleus to drive *Il7ra* expression. PI3K signaling via the pre-BCR can be augmented by CD19 and countered by BLNK(SLP-65). Expression of pre-BCR components is repressed in an IRF4/IRF8-dependent manner to permit Foxo1-dependent expression of *Rag* in resting pre-B cells. Peripheral B cells require PI3K signaling to promote survival while Foxo1 activity is crucial for expression of *Sell* and *Aicda*, suggesting a dynamic balance for Foxo1 activity in peripheral B cell homeostasis and differentiation.

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	Class IA						Class IB		
Gene	*PIK3CA*	*PIK3CB*	*PIK3CD*	*PIK3R1*	*PIK3R2*	*PIK3R3*	*PIK3CG*	*PIK3R5*	*PIK3R6*
Protein	**p110α**	**p110β**	**p110δ**	**p50α, p55α, p85α**	**p85β**	**p55γ**	**p110γ**	**p101**	**p84**
Expression pattern	**broad, elevated in B cells**	**broad, reduced in B cells**	**hematopoietic cells**	**broad**	**broad**	**broad**	**hematopoietic cells**	**hematopoietic cells**	**broad, elevated in heart**
Regulatory or catalytic?	**catalytic**	**catalytic**	**catalytic**	**regulatory**	**regulatory**	**regulatory**	**catalytic**	**regulatory**	**regulatory**