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Review

Critical roles of the PI3K/Akt signaling pathway in T cell development

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Abstract

Thymocyte development requires an integration of extracellular cues to enforce lineage commitment at multiple defined checkpoints in a stage-specific manner. Critical signals from the pre-TCR, Notch, and the receptor for interleukin-7 (IL-7) dictate cellular differentiation from the $CD4^-CD8^-$ (double negative) stage to the $CD4^+CD8^+$ (double positive) stage. The PI3K/Akt signaling pathway is required to translate these extracellular signaling events into multiple functional outcomes including cellular survival, proliferation, differentiation, and allelic exclusion at the β-selection checkpoint. However, a complete understanding of the contributions made by the PI3K/Akt pathway in thymocyte development has not been straightforward. This review highlights studies that support the model that the PI3K/Akt pathway is essential for thymocyte survival. We provide new evidence that Akt-mediated survival is not solely due to the increased expression of Bcl-xL but also is a consequence of the role played by Akt to support metabolism in proliferating thymocytes.

Keywords: Akt; PI3K; Thymocyte; TCR; Bcl-xL; Metabolism

1. Early T cell development

Acquisition of a complete peripheral T cell repertoire requires that T cell progenitors undergo a series of tightly regulated developmental events that depend on integration of signaling cascades downstream of the pre-T cell receptor (TCR) and then the mature TCR. Creating a pool of mature T cells requires that developing thymocytes interpret signals from the extracellular environment in a spatial and temporal-specific manner [1]. The first step in T cell development is the emigration of early thymic progenitors (ETPs) from the bone marrow to the thymus [2]. ETPs then transit through four stages as CD4/CD8 double negative (DN) cells (DN1–4) before upregulating CD8 and CD4 to become double positive (DP) thymocytes [3]. Emergence of mature T cells requires that developing thymocytes pass through several pre-TCR/TCR-dependent selection events: the first at the DN3 stage and then two others at the DP stage. DN3 thymocytes test the newly created pre-TCRβ chain for its ability to be functionally expressed during a process known as β-selection [4,5], while DP cells test the reactivity of the mature TCR for self-peptide/MHC during positive and negative selection [6,7]. This review focuses on the signaling events at the β-selection checkpoint that are required for thymocytes to successfully reach the DP stage.

The diverse repertoire of TCRs expressed on peripheral T cells is the result of the random reassortment of gene sections to create the polymorphic α and β chains. In addition to creating varied TCRs, this random gene rearrangement has the potential to result in proteins that cannot be expressed correctly due to insertion of stop codons or amino acids that preclude proper assembly. The suitability of the TCRβ chain is assessed at the β-selection checkpoint to ensure that only those cells that have rearranged a functional TCRβ chain survive, while thymocytes that fail to produce an appropriately rearranged receptor undergo apoptosis [8]. Survival through this checkpoint requires that cells generate a signal from the pre-TCR, which is composed of the newly rearranged TCRβ chain paired with a non-rearranged pre-Tα chain [9]. Four events occur after successful β-selection: differentiation to the DP stage, proliferation to substantially increase the number of cells with an appropriately created TCRβ

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chain [10,11], prevention of apoptosis through activation of cell survival pathways [8], and allelic exclusion to ensure each cell expresses only a single TCRβ chain [12]. An active area of investigation is to determine how signals that originate from the pre-TCR dictate these four biological events [13].

2. PI3K and PDK1 are required for survival and proliferation at the β-selection checkpoint

Experimental evidence suggests that DN3 cell survival requires that signals delivered by multiple receptors, including Notch, the receptor for interleukin-7 (IL-7), and the pre-TCR, be appropriately integrated [14–17]. However, it remains unclear which signaling pathway(s) is (are) most critical for survival during the transition from the DN3 to DP stage. One pathway common to all three receptors is the phosphatidylinositol 3-kinase (PI3K) signal transduction cascade. Activation of the PI3K pathway supports survival and proliferation of multiple cell lineages [18]. PI3K activation results in the localized increase of phosphorylated lipid second messengers at the plasma membrane [19]. Key signaling intermediates are then recruited to the phosphorylated lipids via specialized lipid-binding domains, pleckstrin homology (PH) domains, and are themselves activated to initiate further signaling events. One key effector molecule that is activated in this manner is the serine/threonine kinase Akt, which, when localized to products of PI3K activation, is able to phosphorylate multiple downstream substrates that mediate cell growth, survival, and metabolism [20,21].

PI3Ks are divided into four classes (IA, IB, II, and III) based on their subunit composition [19]. Class IA and IB PI3Ks are the best understood in the immune system and are the subject of this discussion [22]. Class IA PI3Ks are heterodimers consisting of a regulatory adaptor subunit (p85α, p55α, p50α, p85β, or p55γ) and a catalytic subunit (p110α, p110β, or p110δ) [22]. Class IB PI3Ks differ in their subunit composition, as they are heterodimers of the catalytic subunit p110γ paired with a regulatory subunit p101 or p84 [23]. The catalytic and regulatory subunits exhibit complex patterns of redundant expression. Thus, it has been difficult to use genetically altered mice with single PI3K mutations to unravel the complete role for this signaling pathway in early thymocyte development or subsequent mature T cell function. Genetically modified mice, however, have provided some clues to the importance of PI3K in these events. Although mice deficient in two of the Class IA regulatory subunits, p85α or p85β, develop normal T cells [24,25], mature p85β-deficient T cells engage in more rounds of proliferation and undergo less apoptosis when stimulated *in vitro*, indicating different biological functions for these subunits [25]. Interestingly, the combined loss of both p85α and p85β alter mature T cell signaling, but thymocyte development appears unaffected [26,27], further suggesting that redundancy exists with other less well-defined PI3K regulatory subunits.

Thymic development phenotypes are more apparent in mice that lack functional PI3K catalytic subunits. Although, loss of function of the Class IA catalytic subunit p110δ has no measurable effect on T cell development [28,29], absence of p110γ, a Class IB subunit, results in decreased numbers of DP cells and a corresponding decrease in thymic cellularity [28]. This thymic phenotype correlates with the observation that p110γ$^{-/-}$ DP thymocytes demonstrate increased apoptosis following treatment with anti-CD3 *in vivo*. This TCR-induced apoptosis was not investigated further but is interesting since TCR stimulation normally drives proliferation and survival in wildtype DN3 thymocytes [30]. Mice deficient in both catalytic subunits, p110γ and p110δ, have small thymi due to a combination of increased apoptosis and decreased proliferation at the β-selection checkpoint and DP stages [31,32]. Furthermore, mice that lack both catalytic subunits also demonstrate a paucity of large TCRβ^{+} thymocytes, a population representative of proliferating β-selected thymocytes that have recently received pre-TCR signals [31]. Thus, decreased accumulation of phospholipids at the plasma membrane appears to result in diminished thymocyte survival, likely at the point in which these cells should be undergoing proliferation.

Insights into the role of PI3K in lymphocyte development were further uncovered by studying loss of the lipid phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10), the enzyme that counteracts PI3K [18]. In the absence of PTEN, PH-domain containing kinases, such as Akt, are localized basally to the membrane and become constitutively activated [19]. Activation of these critical signaling effectors initiates a cascade of events and bypasses the requirement for surface receptor engagement, such that loss of PTEN rescues development to the DP stage in mice that are deficient in components of the pre-TCR or the common γ chain cytokine receptor, a subunit required for IL-7 signaling [33]. These data suggest that the activation of the PI3K pathway is sufficient to induce differentiation to the DP stage in the absence of pre-TCR or cytokine signals.

Phosphatidylinositol accumulation by PI3K at the plasma membrane translates into survival and proliferation by triggering the activation of a cascade of serine/threonine kinases. Generally, phosphatidylinositol accumulation induces the localization of PDK1 (3-phosphoinositide-dependent protein kinase 1), a serine/threonine kinase, to the plasma membrane [19]. PDK1 then phosphorylates and activates AGC kinases, such as Akt, S6K1, RSK, and PKC isoforms [19]. The importance of PDK1 as a mediator between PI3K activation and Akt activation is illustrated by the dramatic loss in thymic cellularity and block at the DN4 stage of development in mice that have a conditional deletion in PDK1 [34]. Although the gross phenotype is similar to that of mice deficient in both PI3K catalytic subunits, p110γ and p110δ, the mechanism appears distinct, as thymocytes from these mice did not reveal increased spontaneous apoptosis but rather decreased proliferation [34]. Thus, PDK1 may be more essential for proliferation, while another PI3K-dependent but PDK1-independent pathway signals increased cell survival.

3. Akt is required to maintain thymocyte survival

Given the mounting evidence implicating the PI3K pathway in thymocyte development, several groups began to investigate the importance of Akt, a downstream effector of PI3K activation,