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# Cell Cycle Proteins in B Cell Development: Emerging Links to Vascular Immunity: A Review

Faris Q. B. Alenzi

Ph.D., Professor of Immunology, Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Prince Sattam bin Abdulaziz University (PSAU), Alkharj, Saudi Arabia.
Correspondence: Faris Q. Alenzi, Email: f.alenzi@psau.edu.sa

## ABSTRACT

Cell mitosis and apoptosis proteins (or genes) of different human cell types are required and important to determine the size of the cell mass. Changes above or below the steady state value of 0.5 will result in either expansion or aplasia, respectively. In light of their crucial importance, this article reviews recent developments in the B cell proliferation.

**KEYWORDS**: P27, P130, Cell proliferation, pRB, CDKs, P107, E2F.

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## INTRODUCTION

The retinoblastoma protein (pRB) family of pocket proteins, which includes members pRB, p107, and p130, are the primary physiological substrates of the G1 CDKs[1, 2]. It has been shown that overexpression of any one of the three genes pRB, p107, or p130 can cause cell cycle arrest, which can be overridden by G1 cyclin-dependent kinase activity. Although both the d-type cyclins and cyclin E contribute to pRB phosphorylation in vivo and are equally effective in overcoming a pRB-induced cell cycle block, only the cyclin D·CDK4 complex, but not the cyclin E·CDK2 complex, can phosphorylate p107 and p130 in vivo, dissociate p107 and p130 containing E2F complexes, and effectively alleviate growth suppression by p107 and p130 [3, 4]. These pocket proteins bind to members of the E2F family of transcription factors in their hypophosphorylated forms, negatively affecting the transcription of E2F-regulated genes. The transcription of genes necessary for the cell cycle's S-phase entry depends on E2F activity. E2F activity overexpression can cause cells to advance from G0 through G1 and into S-phase. As a result, G1 cyclins facilitate the pocket proteins' hyperphosphorylation, which results in the derepression of E2F-dependent gene transcription and the passage toward S-phase[5, 6]. Also, The WAF/CIP family of proteins (p21Cip1, p27Kip1, and p57 Kip2), which regulate a wide range of CDKs by binding to the cyclin-CDK complexes, and the INK4 family (p15INK4b, p16INK4a, p18INK4c, and p19INK4d), which specifically inhibit cyclin D-CDK complexes through direct association with CDK[7, 8]. The CKI most closely linked to restriction point regulation is p27KIP1. P27 expression is highly expressed in dormant (G0) cells but is down-regulated as the cells enter the cell cycle in G1.[9] In addition, a number of proteins stop cells from entering the S phase. One group, Rb and the associated proteins p107 and p130, work by rerouting or sequestering transcription factors controlling genes necessary for the S phase[6]. Members of the Ink4 (p15, p16, p18, and p19) and CipyKip families (p21, p27, and p57) operate by inhibiting CDK4y6 and CDK2, two cyclin-dependent kinases[10]. Additionally, these kinases are in charge of coordinating Rb's phosphorylation and inhibiting its growth-suppressive activities. In tumor-derived cell lines lacking Rb, CDK4 activity is not required for S phase entry. However, regardless of Rb status, CDK2 kinase activity is necessary for proliferation, indicating that CDK2 has additional functions necessary for S phase in addition to its role in inactivating Rb[11]. These observations in cell culture indicate that CDK4 and Rb share a regulatory route, but CDK2 controls Rb inactivation and another, as-yet-unidentified pathway. At physiological concentrations, biochemical studies on the interaction of p27 with cyclin D2yCDK4 and cyclin EyCDK2 complexes have revealed that p27 appears to inhibit CDK2 preferentially over CDK4. Based on these observations, we hypothesized that simultaneous p27 and Rb disruption might mimic the CDK2 pathway's constitutive activation and eliminate the need for the CDK4 pathway.[12] However, in numerous cell culture studies, it has been demonstrated that the retinoblastoma (RB) family of proteins (pRB, p107, and p130) controls the activity of members of the E2F family of heterodimeric transcription factors. The RB proteins bind to E2F proteins directly and suppress E2F-mediated transcription; this action is controlled by the phosphorylation of the cyclin-dependent kinase (cdk). Thus, modulation of the numerous E2F family proteins may link the regulation of cell cycle progression to the activation and repression of transcription. A complex web of gene expression programs can be coupled with cell cycle position thanks to fluctuations in the activity of the E2F proteins, which are known to be regulated in a cell cycle-dependent way. Over-expression of E2F or cdks promotes S phase entry, whereas over-expression of the pRB family of proteins causes cell cycle arrest in the G1 phase, according to transfection experiments[13, 14]. It is believed that the effects of pRB family proteins on cell proliferation are mediated by their repressive effects on E2F-driven gene expression because the growth-suppressive functions of the pRB, p107, and p130 proteins map to a region proven to mediate E2F binding[15]. The pRB family of proteins has a repressive effect, at least in part, by attracting histone deacetylase complexes to promoter regions, where they deacetylate histones, causing chromatin to condense and then impede transcription. Also Numerous promoters whose gene products are necessary for cellular processes including DNA synthesis and cell cycle regulation have been studied in over-expression tests for transcriptional activation by E2F and/or repression by E2F-pRB family member complexes.[16] Since induced Bcl-2 expression in dormant fibroblasts leads to raised levels of both p27 and p130, the G1 Cyclin/Cdk inhibitor p27, which inhibits Cyclin E/Cdk2 protein kinase activity, may also play a role in the effect of Bcl-2 on E2F activity. This is supported

by the finding that Bcl-2 expression prevents G1 advancement in cells missing pRb but not p27 or p130, indicating that p130 and p27 are required for Bcl-2's cell cycle inhibitory actions[17]. Given that co-expression of E2F4 enhances the cell cycle inhibitory effects of Bcl-2, the capacity of p130 to form repressive complexes with E2F4 has been suggested.So Bcl-2 expression slows E2F1 accumulation during G1 advancement and overexpression of E2F1 can override Bcl-2 inhibition, the E2F1 gene may be a target of repression by p130/E2F4.25 Therefore, elevated levels of p27 and p130 can account for the Bcl-2-induced G1 arrest mechanism. Increased amounts of p130 would ensure that cells arrest in the G1 phase by maintaining the suppressive p130/E2F4 complex and preventing E2F1 production, while increased levels of p27 would do the same by inhibiting Cyclin E/Cdk2. And It is yet unclear how directly Bcl-2 affects these cell cycle regulators[18] Additionally, it has been shown that animals lacking the cyclin-dependent kinase inhibitor p27 and the pocket protein p130 are unable to effectively generate new blood vessels, impairing their capacity to sustain the growth of tumor xenografts[19]

## THE P13-K SIGNAL TRANSDUCTION CASCADE

Phosphoinositide 3-kinases (P13-Ks) are a collection of lipid kinases responsibe for catalysing the specific phosphorylation of the inositol ring of phosphoinositides at position 3. They are responsible for several cellular responses, including cell growth, survival, metabolism, differentiation, cytoskeletal organisation, and membrane trafficking [20]. The common Class lA P13-Ks are composed of a 110 catalytic and a regulatory subunits of either Kda85, or 55. Recently, the P13-K signalling pathway has been recognized as an important tumor suppressor pathway following the demonstration that the tumour-suppressor PTEN antagonises the functions of the P13-K through conversion of3,4,5-triphosphate (PIP3) into 4,5-triphosphate (PIP2). Furthermore, mutations to PTEN have found in a variety of human cancers, including lymphomas, glioblastomas, melanomas, breast, prostate, thyroid or head and neck cancers. Consistent with this notion, the *pll0α* gene that encodes the catalytic subunit of P13-K, was shown to be frequently enhanced in ovarian or cervical cancers. Moreover, the P13-K regulatory subunit p85 is now reconized to be an oncogene [21]. In addition, Akt, P13-Ks also employ their effects over a wide range of downstream signalling molecules, including mTOR, p70S6 kinases, GSK-3 and the Forkhead family of transcription factors (AFX, FKHR, and FKHR-L1). Despite several studies suggesting that the P13-K pathway plays important roles in proliferation and survival, the signalling events responsible for the proliferative and survival functions of the P13-K pathway remain to be elucidated in addition to understanding the mechanism(s) by which the P13-K pathway connects to the cell cycle and cell death machinery. Engagement of the B cell antigen receptor (BCR) induces a cascade of signaling events that initiates B cell activation resulting in the proliferation of these cells. BCR also plays an important role in the development of B cells, a multi-step progression that results in the differentiation of committed progenitors to terminally differentiated immunoglobulin (Ig) producing plasma cells. In the case of lymphomas it is thought that, this signalling transduction pathway is down-regulated, resulting in uncontrolled growth and survival with unregulated cell differentiation. Autoreactive B cells are eliminated via clonal deletion, occuring primarily at the immature B cell stage. Failure to dispose of unnecessary immature B cells leads to their uncontrolled proliferation and survival that can in turn give rise to the development of autoimmune diseases and cancer [22]. Cross-linking of the BCR on immature WEHI231 B cells resulted in G1 cell cycle arrest and apoptosis. WEHI 231 cells were used as a model for immature B cells and we demonstrated that BCR stimulation results in down-regulation of cyclin D2 and up-regulation of $p27^{(kip1)}$, which was associated with pocket protein hypophosphorylation and E2F inactivation [23] In addition, it has been observed that both cyclin D2 and p27 play roles in regulating survival/apoptosis. These data further suggest that the PI3-K/AKT signalling pathway may mediate the proliferative signals from BCR through cyclin D2 and p27. Investigators examined the mechanisms in which proliferation is controlled in normal and malignant haematopoietic progenitors as well as in other lymphoid cells. It was described common roles and regulatory mechanisms for the pRB pathway, cyclin D, and p27 [24] While engagement of the BCR in immature B cells triggers cell cycle arrest and apoptosis, activation of mature B cells via BCR promotes their proliferation and survival. In these mature B cells, once again demonstrated that cyclin D2 is essential for B CR-mediated pocket protein hyperphosphorylation regulation of E2F activity and S-phase entry. These findings were further confirmed using a D2 'knock-out' mouse model, showing that cyclin D2 is vital for mediating the proliferative functions of BCR and is also important in B cell functions suchas Ig-isotype switching) [25]. Interestingly, deletion of cyclin D2 had no significant effects on T cell proliferation, indicating that the function of cyclin D2 cannot be replaced by other D cyclins in B cells. It was found that the existence of normal B cells in cyclin D2 null mice was owing to the fact that cyclin D3 can partly compensate for the function of cyclin D2 in cyclin D2 deficient B cells [9]. Moreover, while cyclin D2 deficient B cells respond poorly to weak cross-linking of the BCR by monoclonal anti-IgM, they respond normally to LPS. The molecular basis for the differential activation of B cells by IgM and LPS can be attributed to specific targeting of cyclin D2 by IgM. with LPS normally activating both D2 and D3 cyclins [9]. Cyclin D2 deficient mice share phenotypic features with mice that are null for Vav, a Rho-family guanine-nucleotide exchange factor. Defects in B cell activation in response to BCR cross-linking include impaired Ig class-switching, with a significant depletion of CD5+ lymphocytes. These B cell phenotypes are also found in mice defective for factors involved in BCR signalling, including Btk, Lyn, Blnk. Indeed, our recent studies showed that the inability of $vav^{(-/-)}$ B cells to proliferate following BCR ligation is due to a failure to induce cyclin D2 (Giassford et al, unpublished data). Later, it was further demonstrated that intracellular calcium homeostasis is a requirment for cyclin D2 induction, implicating a potential link to the faulty calcium response of $vav^{(-/-)}$ B cells and their inability to induce cyclin D2. Consistent with this, our recent findings also demonstrated that B cells from Btk-/- fail to enhance cyclin D2 expression following BCR cross-linking. Taken together, these findings point to the pRB pathway, in particular cyclin D2, as having an important role in B cell proliferation and development. These results also show that cyclin D2 represents an essential downstream target of the BCR signalling cascade, functioning to integrate BCR signals with the pRB pathway. It is also important to note that a 'linear' axis is evident for the BCR signal transduction pathway, thereby offering potential targets for therapeutic intervention. Although exploration of the signalling events which couple the BCR signals with cyclin D2 and the pRB pathway are important, elucidation of the downstream targets of cyclin D2 in BCR signaling merits further investigation. Previous studies have demonstrated a role for cyclin D2 B cell function, being responsible for the phosphorylation and inactivation of p130. This in turn culminates in release of active E2F followed by entry into the cell cycle. Data from primary and tissue culture B cell systems

demonstrating that p27 functions downstream of cyclin D2 in B cells in response to BCR stimulation. In relation to genetic evidence that p27 functions downstream of cyclin D, a study demonstrated that deletion of *p27* gene restores normal breast and retinal development in cyclin DI null mice, [26]. These findings led us to hypothesis that the pocket protein p130 and/or p27 function downstream of cyclin D2 in B cells. Previous studies using pro-B cells and embryo fibroblasts found that p27 is a target of the P13-K pathway. Inhibition of P13-K by LY294002 or Wortmannin induces an increase in p27Kipl, which then binds to and inhibits the cyclin/CDK2 complexes. In addition, these inhibitors were found to arrest cell growth through inhibition of P13-K and PKB/Akt, activation of the forkhead proteins AFX, FKHR, and FKHR-L1 and up-regulation of p27Kipi expression [27].

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