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Inflammation and Survival Pathways: Chronic Lymphocytic Leukemia as a Model System

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Abstract

A primary response to inflammation is an increased survival of the target cell. Several pathways have been identified that promote maintenance of the cell. The principal mechanism for the extended survival is through induction of anti-apoptotic Bcl-2 family proteins. Bcl-2 was the founding member of this family with five additional members, Bcl-X_L, Bcl-W, Bcl-B, Bfl-1, and Mcl-1, discovered mostly in hematological malignancies. Another mechanism that could add to cell survival is the Pim kinase pathway. This family of enzymes is associated with Myc-driven transcription, cell cycle regulation, degradation of pro-apoptotic proteins, and protein translation. Chronic lymphocytic leukemia serves as an optimal model to understand the mechanism by which these two protein families provide survival advantage to cells. In addition, since this malignancy is known to be maintained by microenvironment milieu, this further adds advantage to investigate mechanisms by which these pro-survival proteins are induced in presence of stromal support. Multiple mechanisms exists that result in increase in transcript and protein level of anti-apoptotic Bcl-2 family members. Following these inductions, post-translational modifications occur resulting in increased stability of pro-survival proteins, while Pim-mediated phosphorylation inhibits pro-apoptotic protein activity. Furthermore, there is a cross talk between these two (Bcl-2 family proteins and Pim family proteins) pathways that co-operate with each other for CLL cell survival and maintenance. Vigorous efforts are being made to create small molecules that affect these proteins directly or indirectly. Several of these pharmacological inhibitors are in early clinical trials for patients with hematological malignancies.

Keywords

CLL; microenvironment; Bcl-2 family proteins; Pim kinases; survival pathways

Introduction

A primary response to inflammation is a need for cells to exist in an environment that is not conducive to cell survival. To create a favorable setting that promotes cell survival and maintenance, microenvironment provides pro-survival factors and mechanisms. While several pathways have been identified, many of these lead to increased production and stability of Bcl-2 anti-apoptotic family proteins. Because chronic lymphocytic leukemia is a

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disease where the leukemic lymphocytes survive due to over-expression of Bcl-2 anti-apoptotic proteins, this malignancy serves as an optimal model system to understand and investigate production and maintenance of Bcl-2 anti-apoptotic proteins. In addition, the expression level of these proteins is augmented when cells are growing in microenvironment niches. These systems, CLL primary cells co-cultured with microenvironment cells such as bone marrow stroma cells, nurse-like cells, CD154-expressing cells in presence of IL-4, act as models for malignant cells growing in bone-marrow, spleen, and lymph node, respectively. We review literature and provide some of our work that uses these model systems. Observations obtained with these systems may be applied to other malignancies or diseases.

Chronic lymphocytic leukemia (CLL)

CLL is presently an incurable disease representing the most common form of leukemia in North America and Europe [1–4]. It is a neoplastic disorder, characterized by a gradual accumulation of small, mature B cells with typical B-cell markers CD5, CD19, CD23, and CD20 [5, 6]. Lack of proliferative properties makes these cells inherently quiescent. However, proliferating pool of cells has been described in lymph nodes and in bone marrow that might feed the accumulating pool in the blood [7, 8]. Even though replicationally dormant, the accumulation of leukemic cells both in the bone marrow and the peripheral blood may be due to the numerous parameters such as, intrinsic defects in their apoptotic machinery or dysregulated production of survival signals from their extrinsic microenvironment.

Bcl-2 anti-apoptotic family proteins as pro-survival members in CLL cells

Bcl-2 anti-apoptotic family

The founding member of this ever growing family of proteins is Bcl-2, B-cell leukemia and lymphoma 2 protein. This is a highly conserved protein throughout evolution and in *C. elegans*, ced-9 has homology to Bcl-2 and it inhibits cell death during worm development [9]. Bcl-2 was discovered by Tsujimoto in Carlo Croce's laboratory in Non Hodgkin's lymphoma cell line with chromosomal t(14;18) [10]. With this translocation, the Bcl-2 gene is under the control of IgH enhancer resulting in over production of Bcl-2 transcripts and protein. McDonnell in Korsemeyer's laboratory elucidated the function of this translocation and protein using Bcl-2 transgenic mice [11]. Bcl-2 has four Bcl-2 homology (BH) domains and a transmembrane domain. Five other members of this family include Bcl-X_L [12], Bcl-B [13], Bcl-W [14], Mcl-1 [15], and Bcl-A1 also known as Bfl-1 [16]. The latter two lack BH4 domain. These anti-apoptotic proteins sequester pro-apoptotic counterparts and a balance between the two determines the fate of a cell [17].

Expression in CLL cells

Studies of CLL have established that the survival advantage of CLL lymphocytes is likely due to aberrant over-expression of anti-apoptotic Bcl-2 family proteins [18–20] in general and Bcl-2 and Mcl-1 proteins in particular. Several lines of evidence support this notion. First, the most consistent cytogenetic lesion in CLL is chromosomal deletions of 13q14 [21], resulting in loss of microRNAs (miR-15, miR-16) that down-regulate Bcl-2 mRNA levels [22, 23]. Second, gene ablation studies in mice have shown that Bcl-2, Bcl-X_L, and Mcl-1 are essential for lymphocyte survival, with different members of the Bcl-2 family playing prominent roles either during early development or later in adult maintenance of mature T and B-lymphocytes [20, 24, 25]. Third, high levels of Bcl-2 and Mcl-1 mRNAs [19] and proteins [26] have been found in CLL, with levels of Mcl-1 inversely correlated with *in vitro* response to chemotherapeutic agents [26]. Fourth, high levels of Mcl-1 are observed in relapsed leukemias [27] and are associated with the failure of CLL patients to achieve

complete responses (CR) to initial therapy with fludarabine [26]. Fifth, down-regulation of these proteins using antisense oligonucleotides or indirect measures to reduce Mcl-1 protein levels results in CLL cell death in culture [28–34]. Sixth, even in clinic, Bcl-2 antisense therapy has shown promising activity in randomized trials [35]. Seventh, Mcl-1 decline in clinical samples show *in vivo* activity against CLL [36]. Finally, microenvironments that mimic bone-marrow and lymph nodes further induce these survival proteins or kinases that affect these anti-apoptotic molecules [37–45]. Taken together, these observations establish anti-apoptotic Bcl-2 family proteins as key survival factors for CLL [46].

Microenvironment impact on chronic lymphocytic leukemia

Chronic inflammation and CLL are inter-related in many aspects. The malfunctioning of the immune system helps the first few cancer cells to establish into a full-fledged CLL. Accumulating evidences imply that clinical manifestations and relentless accumulation of CLL cells are frequently due to the progressive infiltration of malignant B lymphocytes in the bone marrow, lymph nodes, and other tissues [38, 47, 48]. In comparison to normal B cells, leukemic cells are rescued from apoptosis by bone marrow stromal cells, signifying the selectivity of microenvironment for malignant cells. These microenvironmental responses are often brought about by the interplay of different chemokines, cytokines, transcriptional factors or post translational modifications.

Chemokines are classified into inflammatory chemokines [49] which are expressed in inflamed tissues and signal for recruitment of neutrophils and homeostatic chemokines that are produced constitutively in distinct tissue microenvironments to sustain traffic of mature lymphocytes in lymphoid and nonlymphoid tissues [50] . Neutrophiles and monocytes migrate to sites of inflammation in response to chemo-attractants, while stromal cell-derived factor-1, (SDF-1) is a haemostatic chemokine that signals through chemoreceptor CXCR4 and play a functional role in B-lymphopoeisis [51–53]. Other chemokines that are important in this context are CCL3 and CCL4 secreted proteins, which are produced by mature hematopoietic cells(reviewed in [54]) and act as potent chemo attractants for monocyte macrophages, dendritic, T, and natural killer cells [54]. Zucchetto et al and others showed that there was a clear cut over expression of transcripts for CCL3 and CCL4 genes upon CD38 triggering, an anti-apoptotic signaling molecule in CLL [55–57], and blocking CCL3 and CCL4 secretion induced apoptosis in CLL cells [44]. SDF-1 and other chemokines appear to form a pro-survival circuitry by regulating leukocyte trafficking, extravagating into sites of tissue inflammation and maintaining extended lymphocyte survival [58–60].

Cytokines are signaling molecules with variety of cellular functions and are key mediators of inflammation or an immune response. Several of them are involved in accelerating inflammation and are present in high levels in CLL patients. They are classified as pro-inflammatory (IL1, IL6, IL15, IL17, IL23 and TNFα [61]-[62]), or anti-inflammatory (IL4, IL10, IL13, transforming growth factor (TGFβ) and interferon (IFNα)) depending on their function in tumorigenesis. One of the primary function for TNFα is to activate the pro-inflammatory NF-κB, while IL-10 suppresses NF-κB, which leads to reduced levels of pro-inflammatory cytokines TNFα, IL6 and IL12 [63]. IL1, IL6 and TNFα activates the Janus kinase, the activator of transcription signaling pathway, or stimulate IL8/CXCL8 chemokine [64] leading to the increased expression of multiple oncogene [65]. Equally, IL2, IL-6, IL7 and IL21 exhibit pro survival effect on CLL [66–68]. Another potential mediator of inflammation induced carcinogenesis is microRNA and the *miR-15* and *miR-16* on chromosome 13q14 is often deleted in CLL [69]. It has been shown that IL6, a pro-inflammatory cytokine can induce the expression of *miR-21*, an inflammatory stimuli, in a STAT3-dependent manner [70]. TNFα and IFNβ, can stimulate the expression of *miR-155* [71] which is found in increased levels in the bone marrow of leukemic patients and causes hyperproliferation of B-cells [72].

In addition to chemokines and cytokines, the key mediators of inflammation induced cancer include activation of transcription factors. There are a wide range of transcriptional factors that bind to the promoter region of target genes and activate transcription of these oncogenes. Even though *MYC* involved chromosomal translocations are characteristics of human Burkitt's lymphoma, recent studies show that *MYC* rearrangements are associated with complex cytogenetic abnormalities and poor prognosis in CLL [73]. STAT proteins originally shown to exhibit as latent cytoplasmic transcription factors now known to be constitutively phosphorylated on Ser727 and activate transcription in CLL cells [74].

Nuclear factor of κB (NF-κB), that is constitutively activated in CLL [75, 76], is a key mediator of inflammation-induced carcinogenesis [77] and is strongly attributed to interactions of the malignant cells with the bone marrow microenvironment [78]. NFκB activates the transcription of target genes, including inflammation-related genes (e.g. cytokines and chemokines, TNFα, p53 and Bcl-2, Bcl-X_L) [79]. In this respect, Taylor et al proposed a model where they illustrate that integrated effects of three microenvironmental signals (CD40 receptor ligation, IL-4 receptor stimulation and the interaction of integrin ligand VCAM-1 with its receptor) work in concert to increase the mRNA and protein levels of Bcl-X_L and promote Bcl-X_L-Bax binding to suppress drug-induced apoptosis in B lymphoma cells [80]. NF-κB activation could be triggered by members of the TNF super family; for eg. BAFF and APRIL support CLL B-cell survival through activation of the canonical NF-κB pathway [81], or Tcl-1 could activate NF-κB by interacting with p300/ cyclic adenosine monophosphate response element binding protein [82].

Signaling pathways, including those involving NF-κB, MAPK (mitogen-activated protein kinase) and PI3K (phosphoinositide 3-kinase) have been shown to be key regulators of inflammatory cell survival and apoptosis *in vitro*. Manipulation of such pathways *in vivo* has indicated that they also play a role in the resolution of inflammation [83]. ERK inhibitor is shown to enhance the resolution of inflammation while BAX inhibitor delayed the inflammation resolution [84, 85].

In conjunction with transcriptional regulation, post translational modifications of Bcl-2 family proteins further affect longevity of anti- and pro- apoptotic proteins [40]. Bcl-2 protein undergoes phosphorylation at sites Thr56, Thr69, Ser70, Thr74 and Ser87 in response to different stimuli. Bcl-2 phosphorylation at Ser70 has been found necessary for its potent anti-apoptotic function [86–89] and associate with poor survival in AML [90, 91]. Bcl-2 phosphorylation regulates its interaction with BAX, BIM or BID [91] and protects Bcl-2 from proteasomal degradation and increase stabilization [92, 93].

Another important anti-apoptotic Bcl-2 family protein, Mcl-1 has an extended amino terminal PEST region [15] and AU rich elements [94, 95] in the 3' – untranslated region, which are responsible for its relatively short half life, and it has two caspase cleavage sites D127 and D157 for faster degradation [96]. However, it has several mechanisms for increased production and maintenance of protein, which are depicted in part in Figure 1. Activation of c-Myc and NF-κβ induce transcription of Mcl-1. It is also rapidly transcribed via a PI3K/AKT-dependent pathway, resulting in its increased expression during myeloid differentiation and cytokine stimulation [97–99], and it is phosphorylated upon oxidative stress and cytokine withdrawal [100, 101]. Increased transcript levels lead to higher protein levels and proteins such as Pim kinase (induced by microenvironment) and 4E-BP1 assist in this pathway (Figure 1, pathway II). Phosphorylation at Thr163, the conserved MAP kinase/ ERK site located within PEST region, slows Mcl-1 protein turnover (Figure 1, pathway III) [102], but may prime the GSK-3β mediated phosphorylation at Ser159 that leads to Mcl-1 stabilization (Figure 1, pathway I) [103]. Microenvironment-mediated phosphorylation by Akt inactivates GSK-3β resulting in inhibition of proteasomal degradation of Mcl-1.

Microenvironment factors also stimulate JNK pathways, and JNK1 phosphorylates Mcl-1 at Ser64 resulting in increased Mcl-1 anti-apoptotic function and binding and sequestering pro-apoptotic proteins (Figure 1, pathway IV) [104, 105]. Finally Mcl-1 has a selective ubiquitinating and deubiquitinating enzymes, MULE [106] and USP9X [107] respectively. In contrast to Mcl-1, Bcl-2 is a long-lived protein [108].

Phosphorylation of pro-apoptotic protein Bad via PI3K/AKT and Pim kinase pathways leads to its premature degradation and survival of cancer cells. This will be further discussed in the Pim kinase section of this review. Taken together, the extracellular signals from cytokines and chemokines, the contribution of transcriptional factors and post-translational modifications on anti-apoptotic proteins ultimately form a complex network to deliver microenvironmental support to the malignant cells.

Targeting Bcl-2 survival pathways

The recent advancement in understanding the primary reasons for extended survival of CLL cells had proposed many strategies to inhibit anti-apoptotic proteins in this tumor model. One key approach is to employ synthetic BH3 mimetics to modulate the function of Bcl-2 [109]. Bcl-2 inhibitors are synthesized in a manner that they mimic the structure and/or function of BH3 domain of the pro-apoptotic proteins and competitively bind to anti-apoptotic proteins with greater affinity to displace the sequestered pro-apoptotic proteins.

Obatoclax, an indole bipyrrole compound was specifically designed to inhibit all relevant anti-apoptotic members of the Bcl-2 family (Bcl-2, Bcl-X_L, Mcl-1, Bcl-W, Bcl2-A1, and Bcl-B) with an IC_{50} of 1–7 μM [110] . Preclinical studies with obatoclax revealed promising results in multiple myeloma [111], acute myeloid leukemia [112] and mantle cell lymphoma [113] and solid tumors [114, 115]. It is first in its class of BH3 mimetics to enter clinical trials [116].

Polyphenol compound gossypol is a natural product BH3 mimetic, designed to inhibit all 3 major anti-apoptotic proteins, Bcl-2, Bcl-X_L and Mcl-1 with Ki values of 35 nM , 600 nM and 25 nM respectively[109]. Our investigations using CLL primary cells revealed that gossypol, a BH3-mimetic, induces caspase independent apoptosis; required Bax recruitment to the mitochondrial membrane to trigger cytochrome c and AIF release into the cytosol [117]. Although gossypol was tested clinically [118]-[119], mainly this compound has been considered a lead compound for the development of a new class of antineoplastic agents.

AT-101 is ℓ-isomer of gossypol, developed as more potent, less toxic compound compared to gossypol with added oral bio-availability. Similar to gossypol, AT-101 binds to the BH3 motif of all major anti-apoptotic proteins, besides with greater affinity than the parent compound (e.g., 230, 570, and 130 nM for Bcl-2, Bcl-X_L, and Mcl-1, respectively) [120]. Extensive studies with single agent AT-101 in CLL demonstrated promising results [120, 121] and its combination with monoclonal antibody rituximab exhibited synergy [122]. Additionally, AT-101 by far overcame the protective effect conferred by nurse like cells in CLL [120] or bone-marrow stroma cells [121]. Phase I study of single agent AT-101 in advanced malignancies [123] and an open-label phase II study to evaluate the safety and efficacy in combination with rituximab in patients with relapsed or refractory CLL [124] have been closed, however, phase I randomized study in combination with chemotherapy or chemoradiotherapy in solid tumors are ongoing.

The ABT-737, is a small molecule Bcl-2 inhibitor, designed to mimic BH3 domain of BAD protein [125]. In comparison to other Bcl-2 inhibitors, ABT-737 displays high potency, with superior affinity to Bcl-2, Bcl-X_L, and Bcl-W (K_i, <1 nmol/L for all three proteins) [126]. Unlike other pan Bcl-2 inhibitors, ABT-737 has a constraint that it does not bind to other

members in the Bcl-2 family, such as Mcl-1 or A1 [127]. In recent years, ABT-737 has been studied widely in many types of cancer systems. This compound exhibits anti-tumor activity in broad range of tumor models including solid tumors [126] and hematological malignancies [128–130]. Mechanistically, this compound primarily induces apoptosis through mitochondrial pathway, the hallmark feature of intrinsic apoptotic pathway by disrupting Bcl-2/Bax association [128] or dissociating Bim from Bcl-2 [131]. Thus, ABT-737 classically represents the function of a BH3 only protein either by activating pro-apoptotic Bcl-2 proteins (Bak/Bax) or by inhibiting anti-apoptotic protein functions (Bcl-2/Bcl-X_L). In CLL cells, ABT-737 showed nanomolar efficacy (EC_{50} of 4.5 ± 2.2 nM) [129] in displacing Bim from Bcl-2 to promote cell death, signifying Bcl-2 complexed to Bim is the critical target for ABT-737 in CLL [129].

Pim Kinases as pro-survival members in CLL cells

Pim kinase family

While targeting Bcl-2 family proteins is one strategy, the other is to identify Pim kinases as pro survival members of CLL cells. Proviral integration of murine leukemia viral (Pim) kinases were discovered in 1984 [132], and are anti-apoptotic serine/threonine kinases. Increased levels of these proteins have been implicated in transcription regulation, cell survival, tumorigenesis/leukemogenesis and resistance to cytotoxic agents (reviewed by Amaravadi [133]), and Pim kinases have been identified to be over-expressed in leukemias (reviewed by Shah[134]). Three Pim kinases have been identified to date (Pim-1, -2 and -3), each with variant isoforms of expressed protein due to alternative translational start sites [135]. At the amino acid level, there is substantial homology between Pim-1 and Pim-2 (53%) [136] and Pim-3 (69%) [137]. The three proteins have overlapping functions and compensate for one another and Pim-1 knockout experiments identified high frequency of activation of Pim-2 [138].

Function

Pim kinases are constitutively active and their activity affects a number of cellular processes [139–141]. An ever-increasing number of proteins have been identified as phosphorylation targets by Pim-1, and a selection of targets and their functions are shown in Figure 2. Phosphorylation of Histone H3 at Ser10 by Pim-1 is necessary for *MYC*-dependent transcriptional activation and oncogenic transformation.[142] In addition, c-Myc phosphorylation at Ser62 by Pim kinases results in the stabilization of c-Myc protein.[143] These actions support the generation of oncogene transcripts and the sustenance of oncogenic protein levels. Many of c-Myc-target oncogenes have short transcript half-lives and thus inhibition of Pim kinase activity would reduce the expression levels of these oncoproteins. *MCL-1* is one such oncogene (Figure 1, pathway II) , and disruption of Pim function may be instrumental in killing cancer cells whose survival is dependent on such oncogenes (reviewed in [144]).

The survival advantage provided by Pim kinases is not limited to driving the expression of *MYC* target genes, but also via the disruption of apoptotic signaling pathways. *PIM1* has been shown to efficiently cooperate *BCL2* genes in lymphomagenesis,[145] and the simultaneous upregulation of Pim-1 and anti-apoptotic protein A1 is required for Bcr-Abl-induced leukemogenesis [146]. Pim kinases are involved with the regulation of pro- and anti-apoptotic Bcl-2 family proteins, and one of the targets of Pim kinase phosphorylation is pro-apoptotic Bad protein. All three Pim kinases phosphorylate Bad with varying specificities for the different phosphorylation sites (Ser112, Ser136 and Ser155) [139, 147], however the predominant target is the Ser112 “gatekeeper” site [148]. Bad protein phosphorylation at Ser112 by Pim-1 enhances phosphorylation at Ser136 by other kinases

such as Akt[148]. Phosphorylation of Bad protein allows binding to 14-3-3 scaffold protein, which prevents 14-3-3 binding to anti-apoptotic Bcl-2 or Bcl-X_L proteins and promotes cell survival [149, 150].

Regarding cell cycle regulation, Pim kinases have been shown to phosphorylate cell cycle proteins such as cyclin-dependent kinase (CDK) inhibitors p21 and p27, and phosphatases cdc25a and cdc25c. Pim-1 function on p21 may contribute to tumorigenesis in part via stabilization of p21 and subsequent promoting cell proliferation. Thr145 site on p21 is a Pim-1 target, and its phosphorylation results in the stabilization of p21 protein and disruption of the association between proliferating cell nuclear antigen (PCNA) and p21 [151]. p27 is also a CDK inhibitor of abnormal cell cycle progression, and Pim kinases promote cell cycle progression and tumorigenesis via the down-regulation of p27 expression at both the mRNA and protein level [152]. Pim-mediated suppression of *p27* transcription is via the phosphorylation and inactivation of forkhead transcription factors, FoxO1a and FoxO3a. All three Pim kinases can phosphorylate p27 at Thr157 and Thr198, which as with Bad protein, allows binding to 14-3-3 protein resulting in nuclear export and proteasome-dependent degradation. Similarly, both cdc25a and cdc25c are substrates of Pim kinases, and phosphorylation of these cell cycle phosphatase positively regulate cell cycling [153, 154]. In addition to cell cycle effects, Pim kinases may contribute to proliferation via regulation of protein translation. Pim-2 phosphorylates translation repressor protein 4E-BP1 at Ser65, which is the site required for inhibition of binding to eIF4E and formation of the active translational initiation complex [135, 155, 156]. Phosphorylation at Ser65 also inhibits 4E-BP1 pro-apoptotic activity [157], and thus Pim kinase induction of cell survival signaling may be in part through 4E-BP1.

Degradation

Pim kinases interact and phosphorylate SOCS1 and SOCS3 [158, 159], which are negative regulators of STAT-driven Pim expression. The stability and function of Pim-1 is further regulated in part by phosphatase PP2A [160] and heat-shock proteins (HSPs) and through the ubiquitin-proteosome pathway [141, 161]. Through interaction with PP2A, dephosphorylation of Pim-1 allows for ubiquitinylation and protein degradation of Pim-1 [160]. With respect to HSPs, in *PIM1*-transfected BAF-B03 cells, an interleukin 3 (IL-3)-dependent murine pro-B-cell line, both HSP90α and HSP90β were identified to interact with Pim-1. Furthermore, treatment with HSP90 inhibitor, geldanamycin, resulted in the reduction in both Pim-1 kinase activity and protein stability [161]. Pim-1 is tagged with ubiquitin by HSP70 for degradation by the proteosome, whereas HSP90 binding of Pim-1 provides protection from proteosome degradation [141].

Hypoxic conditions can also alter Pim-1 expression and degradation, and hypoxia increases Pim-1 in several cancer cell lines in a hypoxia-inducible factor-1alpha-independent manner [162]. In pancreatic cancer cell line PCI-43, hypoxic conditions result in the stabilization of Pim-1 via prevention of its ubiquitin-mediated proteasomal degradation [163]. This increase in Pim-1 protein contributes to solid tumor progression and hypoxia-induced chemoresistance, and thus Pim-1 may act as a survival factor under hypoxia.

Microenvironment impact on Pim kinase expression

Pim kinases are transcriptionally regulated and are targets of a variety of growth factor-dependent transcriptional responses [135, 164]. Stromal cell co-culture results in STAT5 activation and increased expression of *PIM1* transcript [165], and IL-5 enhances gene expression of *MYC*, and *PIM-1* in activated B cells [166]. In leukemic cells, previous studies in our laboratory and other groups have shown that Pim-2 RNA and protein levels are higher in primary CLL cells compared with normal lymphocytes [31, 167]. The

mechanism of increased Pim-2 expression in CLL is unknown, but its increased expression may provide a survival advantage to CLL cells. Microenvironment factors may play a key role in upregulation of Pim kinases and interestingly, Pim kinases themselves may provide a feedback loop as recent advances have shed light on Pim kinase function on microenvironment signaling. CXCR4 is phoshorylated by Pim-1 at Ser339, a site known to be essential for normal receptor recycling [168].

The proposal of CLL primary cells co-cultured with microenvironment cells as a model system for inflammation is supported by investigations in various other hematopoietic cell types that are involved with inflammation. In eosinophils, Pim kinases are involved with inflammation induced by allergic response. Allergic inflammation is characterized by elevated eosinophil numbers and by the increased production of the cytokines IL-5 and GM-CSF, which control several eosinophil functions, including the suppression of apoptosis. Treatment of human eosinophils with IL-5 or GM-CSF results in the activation of STAT3 and STAT5 signaling, and as described earlier, Pim-1 is a target gene of STAT5. This signaling activation induced the protein expression of cyclin D3 and Pim-1, and Pim-1 action is linked to the suppression of eosinophil apoptosis by these cytokines [169]. In basophils, the contribution of these cells in allergic disease depends on their persistence at sites of inflammation (reviewed in [170]) Basophils have a considerably longer spontaneous life span than neutrophils and eosinophils [171, 172], and IL-3 protects basophils from apoptosis through the upregulation of the protein expression of a number of anti-apoptotic proteins including cIAP2, Mcl-1, and Bcl-X_L and Pim-1, which is correlated with enhanced cell survival [171].

Targeting Pim kinase pathways

The therapeutic strategy of Pim kinase inhibitors are to block critical signaling pathways involved with cell survival or proliferation to induce cell death in malignant cells. Pim kinase overexpression in malignant cells renders them more susceptible to Pim kinase inhibition, and thus may also be a method to limit toxicity to normal healthy cells. There are currently a number of Pim kinase inhibitors at the preclinical development stage, including a recent report on SGI-1776 from our laboratory [31]. Our studies demonstrated that SGI-1776 was preferentially cytotoxic in towards primary CLL cells compared with normal lymphocytes from healthy donors, and resulted in the decrease of both Mcl-1 protein and transcript level in treated cells. Other Pim inhibitors from both academic institutions [173–175] and pharmaceutical companies are also under development (reviewed in [176]) with varying levels of specificity towards Pim kinases. There has also been a recent report describing the development of monoclonal antibodies against cell surface Pim-1 [177]. The use of Pim kinase inhibitors is in an early stage in development, however, may be a valuable new class of therapeutics for the treatment of cancers.

Conclusion

The pathway similarities between CLL cultured in the microenvironment and inflammation leads to speculation of whether BH3-mimetics or Pim kinase inhibitors may have anti-inflammatory properties. Furthermore, because there is a cross-talk between these two pathways, a combination of two could be explored. Since prevention is a favorable option for inflammation, these agents could also be investigated for such potentials.

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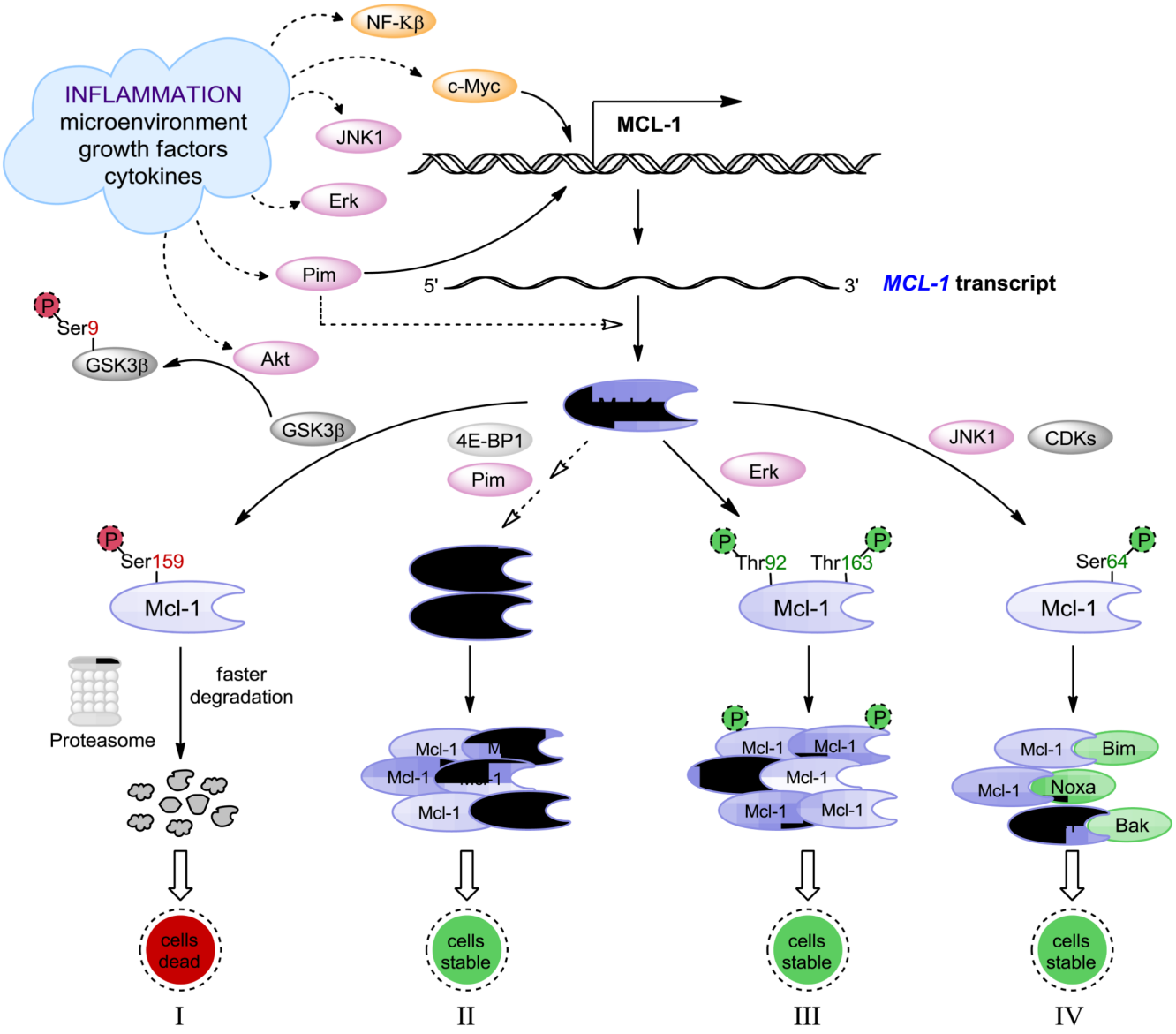

Figure 1.

Microenvironment-mediated transcription, translation, and post-translational modifications of Mcl-1 anti-apoptotic protein. (I) Accelerated degradation of Mcl-1 protein following phosphorylation at Ser159 by GSK-3. (II) Increased Mcl-1 protein levels from Pim/c-Myc driven gene expression. (III) Mcl-1 protein stabilization via phosphorylation at Thr92 and Thr163 by ERK. (IV) Increased Mcl-1 anti-apoptotic function mediated by phosphorylation at Ser64 by JNK/CDKs resulting in increased binding and sequestering of pro-apoptotic proteins.

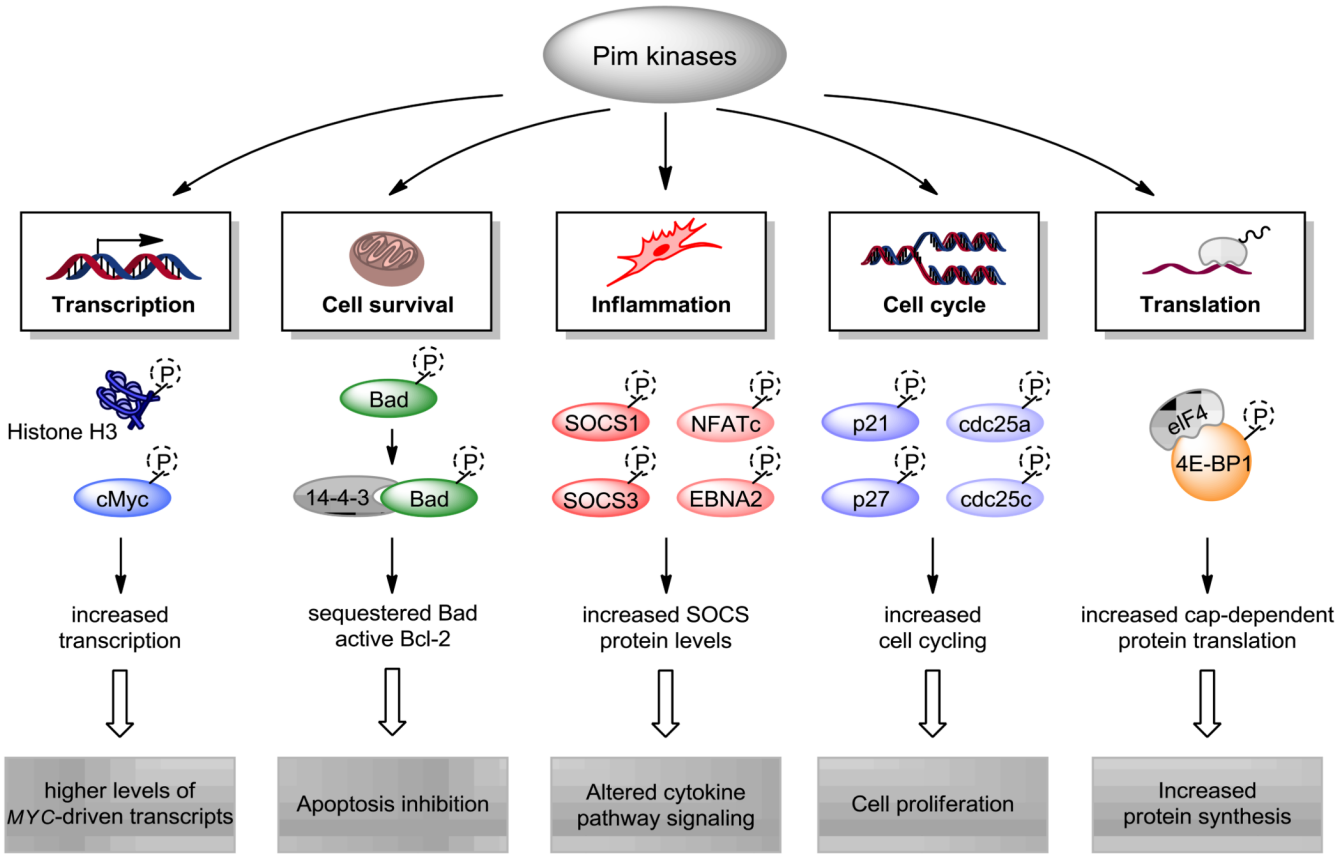

Figure 2.

Pim kinase targets and their roles in inflammation and cancer. Pim kinases phosphorylate a number of substrates such as histone H3 and c-Myc, which activate Myc-driven transcription. Apoptotic protein targets of Pim kinases include pro-apoptotic Bad protein, and Bad phosphorylation allows binding with scaffold protein 14-3-3 and the inhibition of apoptosis. In the inflammatory response, Pim phosphorylation of various targets results in stabilization of SOCS proteins, which leads to cytokine pathway signaling. Cell cycle (p21, p27, cdc25a and cdc25c) protein phosphorylation removes cell cycle checkpoints and induces proliferation. Cap-dependent protein translation is increased by phosphorylation of 4E-BP1, a Pim kinase target. Collectively, phosphorylation of these Pim kinase targets results in increased transcription, protein synthesis, inflammatory response, cell survival and proliferation.