Regulation of quiescence in lymphocytes

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Cellular quiescence is a state characterized by decreased cell size and metabolic activity. In quiescent naïve lymphocytes, antigen recognition with appropriate co-stimulation triggers exit from G0 phase of the cell cycle, increased size and metabolism and progression through the cell cycle. Recent studies have shown that quiescence in lymphocytes is an actively maintained state rather than a default pathway in the absence of signal. Certain transcription factors appear to act as master regulators of gene expression patterns that enforce the quiescent phenotype. Here, we discuss candidate lymphocyte quiescence factors and their potential target genes and mechanisms of action.

Cell-cycle exit from G1 into G0 phase (quiescence) is accompanied by lower rates of transcription, translation, metabolism and reduced cell size. Presumably, quiescence in naïve lymphocytes acts to reduce the resources (energy and space) required to maintain a vast repertoire of T and B cells, only a small fraction of which will be clonally selected by antigen during the lifetime of the host. Quiescence might also protect cells from accumulating metabolic damage as well as genetic changes that could result in malignancy. Quiescence is a distinct state from anergy in that cells are responsive to activating stimuli and resistant to apoptosis (Table 1). Although other lymphocyte subsets (e.g. memory) can have characteristics of quiescent cells, this Review will focus on naïve T and B cells.

One might imagine that quiescence results simply from the absence of activation signals that induce the cell growth and division machinery. However, there is growing evidence that quiescence is under active transcriptional control. For example, DNA microarray experiments have shown that activation of T and B cells involves not only increased expression of genes promoting growth but suppression of a 'quiescent' gene expression program [1–3]. Global expression data have also provided a useful starting point for choosing candidate quiescence factors. Transcriptional control of cellular and organismal quiescence appears to be an evolutionarily conserved phenomenon because a single transcription factor [*DAF-16* (Dauer-formation mutant 16)] is required for the nematode *Caenorhabditis elegans* to enter a quiescent developmental state known as dauer arrest.

There are many proteins that can inhibit lymphocyte activation and/or cell-cycle progression. These include signaling components that negatively regulate activation signals as well as cell cycle inhibitor proteins. In this Review we make a distinction between those proteins that negatively regulate a crucial step in cell-cycle progression, leading to cycle arrest and/or apoptosis and transcriptional regulators that promote a broad gene expression program that programs a quiescent state. Transcription factors that promote quiescence, a reversible cell fate, are also distinguished here from factors (e.g. polycomb group) that determine irreversible cell differentiation decisions in lymphocytes [4].

Investigating the molecular switches that regulate lymphocyte quiescence will enable us to gain a better understanding of cell-cycle control in normal lymphocytes. Furthermore, quiescence factors represent a potential class of tumor suppressor genes because alterations in their expression or function might contribute to progression of lymphoid malignancies. Thus, strategies to enforce quiescence might be useful in controlling leukemia and lymphoma. To illustrate these concepts, we begin by reviewing studies of the transcriptional regulator, lung Krüppel-like factor (LKLF).

LKLF and T-cell quiescence

Krüppel-like factors (KLFs) bind to GC-rich DNA elements through conserved DNA-binding zinc-finger domains in the C-terminus [5]. The unique functions of KLFs derive mainly from N-terminal domains involved in transcriptional activation or repression. In addition, KLFs are expressed in a tissue-specific and regulated manner. The expression pattern of *LKLF* is consistent with a possible role in quiescence: it is highly expressed in naïve T cells, rapidly lost after T-cell activation [1,2] and is re-established in memory T cells, another quiescent population [6].

Elegant work has demonstrated that *LKLF* is both necessary and sufficient to program quiescence in T cells. Kuo *et al.* assessed the effect of *LKLF* gene disruption on development and function of normal T cells using *RAG-2* (recombination-activating gene 2)-deficient blastocyst complementation because germline knockout mice die as early embryos [7]. *LKLF*-deficient RAG-2 chimeras did not show any significant alteration in thymocyte development, yet few T cells were present in the lymph nodes and there was a complete absence of T cells in the peripheral blood [8]. The few lymph-node T cells that could be recovered showed an activated phenotype and proliferated

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Table 1. Properties of lymphocyte differentiation states[a]

Phenotype	Quiescent lymphocytes	Activated lymphocytes	Anergic lymphocytes
Cell size	Small	Large	Large
Rate of protein synthesis	Low	High	Low
Response to activation	Proliferation	Expansion or apoptosis	None or apoptosis
Sensitivity to FasL-dependent apoptosis	Low	High	High

[a]Abbreviations: Fas L, Fas ligand.

spontaneously [8]. However, these activated T cells were rapidly eliminated by Fas-mediated apoptosis. These findings are consistent with the idea that LKLF is necessary for maintenance of T-cell quiescence but that expansion of cells lacking this quiescence factor is prevented by activation-induced cell death. It would be interesting to determine if overexpression of an anti-apoptotic protein, such as Bcl-X_L, in the *LKLF*-deficient background would lead to autoimmune pathology and/or increased incidence of T-cell leukemia. Such a finding would support the idea that altered quiescence-factor function could influence the development of immune disorders and cancer.

To determine if LKLF expression alone could program quiescence in a transformed T cell, Buckley *et al.* generated stable transfectants of the *LKLF* cDNA under the control of a doxycycline-inducible promoter [9]. LKLF expression in Jurkat cells did not induce apoptosis but caused a marked decrease in cell size, proliferation, metabolic rate and expression of cell-surface transferrin receptor (CD71), an activation marker. These effects were reversible on the removal of doxycycline. These data illustrated that overexpression of a single transcription factor, LKLF, could switch a leukemia cell into a dormant state.

If LKLF is a master regulator of quiescence, what are its crucial target genes? Buckley *et al.* addressed this question using DNA microarray analysis of gene expression in Jurkat transfectants cultured in the presence or absence of doxycycline [9]. This experiment revealed the proto-oncogene *Myc* to be a target gene of LKLF (Fig. 1). LKLF overexpression reduced *Myc* mRNA levels by 90% and protein levels were similarly diminished [9]. These effects were dependent on the transcriptional activity of LKLF because the N-terminal-deleted mutant failed to induce *Myc* downregulation [9]. This implies that LKLF induces expression of a factor that suppresses *Myc* transcription.

Myc is a transcription factor involved in controlling cell growth, cell cycle progression, differentiation and apoptosis [10] (Fig. 1). One of the targets of Myc is *cdc25a*, a phosphatase involved in activating cyclin-dependent kinase 2 (cdk2) (reviewed in Ref. [11]). In addition, Myc might repress expression of *Gadd45* (growth arrest and DNA damage-induced gene 45, a growth-inhibitory gene; Fig. 1) because Myc-deficient cells have elevated *Gadd45*

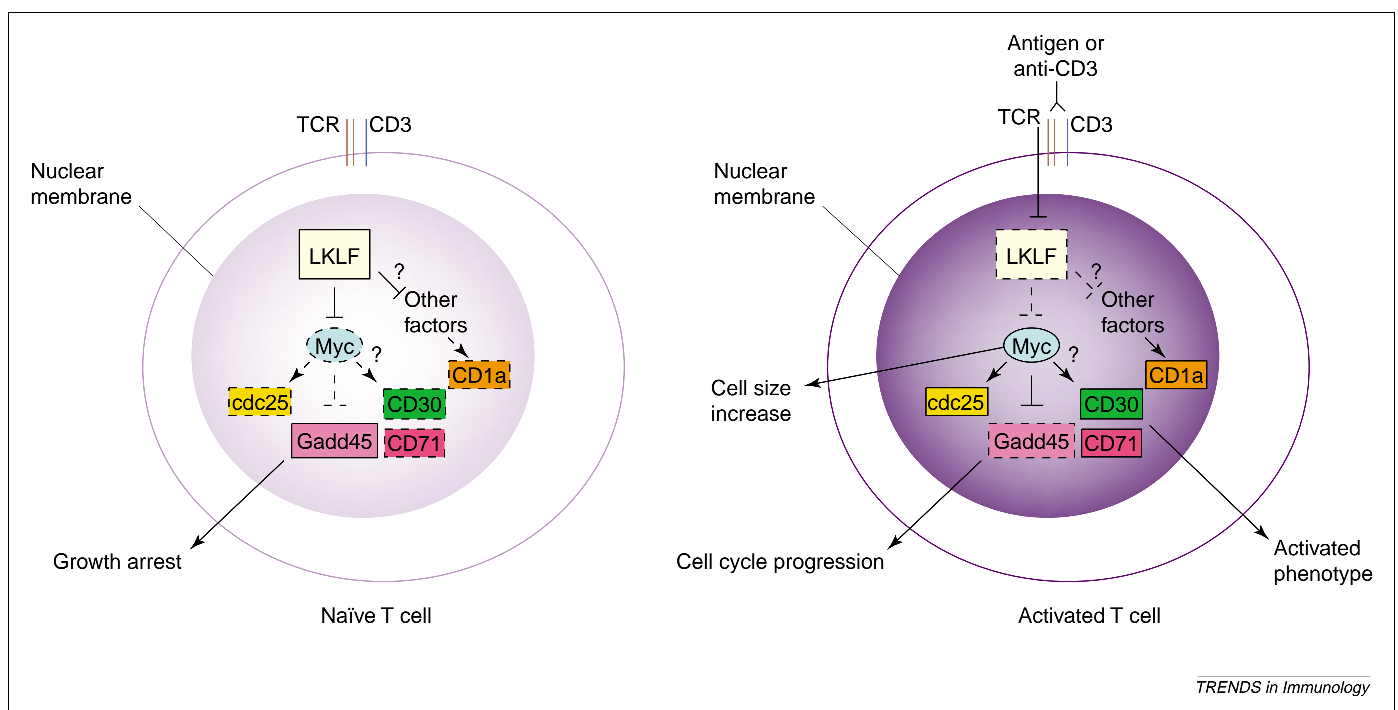

Fig. 1. Role of lung Krüppel-like factor (LKLF) in programming T-cell quiescence. LKLF promotes a quiescent phenotype by downregulating the expression of the proto-oncogene, *Myc.* Myc promotes cell cycle progression by upregulating genes, such as *cdc25,* which encodes a phosphatase shown to activate cyclin-dependent kinase-2 (cdk-2). Myc also downregulates the expression of *Gadd45* (growth arrest and DNA damage-induced gene 45), which has growth inhibitory affects. Expression of Myc in LKLF-deficient cells increases the levels of CD30 and CD71, but not CD1a, suggesting that LKLF promotes quiescence through other transcription factors in addition to Myc. Finally, Myc has also been shown to promote cell size increase in B cells. Unbroken lines indicate function or expression. Broken lines indicate loss of function or expression. Abbreviation: TCR, T-cell receptor.

expression [12]. Overexpression of Myc or mutations in its coding sequence has been implicated in B-cell malignancies (reviewed in Ref. [11]). In lymphocytes, *Myc* expression is associated with both proliferation and increased cell size [9,13]. All these lines of evidence support a probable link between Myc downregulation and the quiescent phenotype of cells overexpressing LKLF.

Direct evidence for this model was obtained from experiments in which either Myc or a dominant-negative form, MadMyc, were transfected into Jurkat cells. Co-expression of Myc blocked the ability of LKLF to decrease cell size and reduce expression of the activation markers CD71 and CD30. Conversely, expression of MadMyc alone decreased cell size and expression of CD71 and CD30 [9]. Interestingly, expression of Myc or MadMyc did not influence the expression of CD1a, another activation marker downregulated by LKLF. This suggests that downregulation of Myc is not the only mechanism by which LKLF programs quiescence in this system (Fig. 1). One possibility is that the Myc-suppressing factor induced by LKLF expression also suppresses other genes. LKLF could also directly activate transcription of genes involved in limiting metabolism and other hallmarks of quiescence. Some clues could be provided by further analysis of microarray data from the LKLF induction experiment. Nonetheless, the Myc experiments emphasize that quiescence factors act as master regulators in part by regulating the expression of other transcription factors.

There is growing evidence that other KLF family members have growth-suppressive properties in various cell contexts. Forced expression of EKLF (erythroid KLF) in immortalized fetal-liver erythroblasts inhibited proliferation [14]. Similarly, induced expression of GKLF (gut-enriched KLF) in a human colon-cancer cell line inhibited growth [15]. Finally, *KLF6* has been defined as a tumor suppressor gene mutated in human prostate cancer [16]. A microarray study by Teague *et al.* found that, like LKLF, EKLF and BKLF (basic KLF) are expressed in resting T cells and downregulated following activation [2]. However, the spontaneous activation of LKLF-deficient T cells implies that EKLF and BKLF are insufficient to maintain quiescence in primary cells. Whether EKLF or BKLF overexpression can enforce quiescence in leukemia cells has not been tested.

KLFs in B cells

Although factors that enforce quiescence in B cells have not yet been established, it seems probable that quiescence in B cells is actively maintained. In support, microarray experiments have revealed that activated B cells downregulate expression of a large group of genes. Of note, the KLF family members LKLF, GKLF and BKLF are all expressed in resting murine B cells and downregulated following activation through the B-cell receptor (BCR) [3,17]. We have confirmed these patterns of mRNA expression by RT–PCR (I. Yusuf *et al.* unpublished). LKLF itself does not seem to be required for B-cell quiescence because LKLF-deficient B cells were not spontaneously activated [8]. Among KLF family members, LKLF is most homologous to GKLF. Considering the established growth-inhibitory properties of GKLF [15], a reasonable model is that GKLF functions to maintain B-cell quiescence, either alone or together with LKLF. GKLF-deficient mice are born but die within 24 h owing to the loss of skin barrier function [18]. Hence, analysis of GKLF-deficient B cells awaits RAG-2 complementation or fetal liver transfer experiments. The ability of any KLF family member to promote quiescence in B-cell tumor lines has not been tested.

Tob

Another transcriptional regulator suggested to be a quiescence factor in T cells is Tob. Tob is highly expressed in anergic T cells as well as naïve peripheral-blood $CD4^+$ T cells [19]. Further, Tob expression was almost completely shut-down when T cells were activated by T-cell receptor (TCR) crosslinking in the presence of CD28 co-stimulation or interleukin-2 (IL-2) receptor-mediated signals [19]. The effects of forced Tob expression in activated T cells were tested by a protein transduction strategy. $CD4^+$ T cells transduced with Tob showed reduced production of cytokines, such as IL-2, interferon-γ (IFN-γ), IL-4 and IL-10. Conversely, antisense-mediated inhibition of Tob expression lowered the TCR stimulation threshold for activation and triggered increased production of IL-2, IFN-γ and IL-4 [19].

Tob is a member of a gene family [recently termed *APRO* (anti-proliferative) genes] previously implicated in growth suppression [20]. Little is known about their molecular mechanism of action or whether they regulate target-gene expression. A clue to the mechanism of Tob function came from experiments demonstrating its ability to interact with Smad proteins. Members of the Smad family of transcription factors function downstream of receptors for transforming growth factor-β (TGF-β) family ligands (Fig. 2), either increasing or suppressing transcription. Tob interacts preferentially with Smad2 and Smad4. Tob enhanced the ability of Smad4 to bind to a consensus DNA binding element and augmented Smad4 transcriptional activity in a reporter gene assay [19].

In T cells, a crucial function of Tob–Smad complexes could be to directly suppress *IL-2* gene transcription (Fig. 2). A Smad-binding DNA element encompasses position -105 in the IL-2 promoter. In cotransfection experiments in the Jurkat cell line, Tob expression increased the ability of Smads to antagonize IL-2 promoter activation, dependent on an intact Smad site [19]. In keeping with blockade of cytokine production, Tob overexpression in primary T cells led to a pattern of cyclin-cdk expression consistent with an arrest in G1 phase rather than G0. It has not yet been determined whether Tob and/or Smads interact with the promoters of *IL-4* or *IL-10*, whose expression is also suppressed in T cells transduced with Tob (Fig. 2). Analysis of endogenous transcription-factor complexes in normal T cells will be necessary to provide further support for the model that Tob and Smads interact to bind target promoters.

Based on these overexpression and antisense experiments, Tob does not fit the strict definition of a quiescence factor. Overexpression appears to induce cell-cycle arrest in G1 rather than G0. Furthermore, reduced expression lowers the TCR signaling threshold for productive T-cell

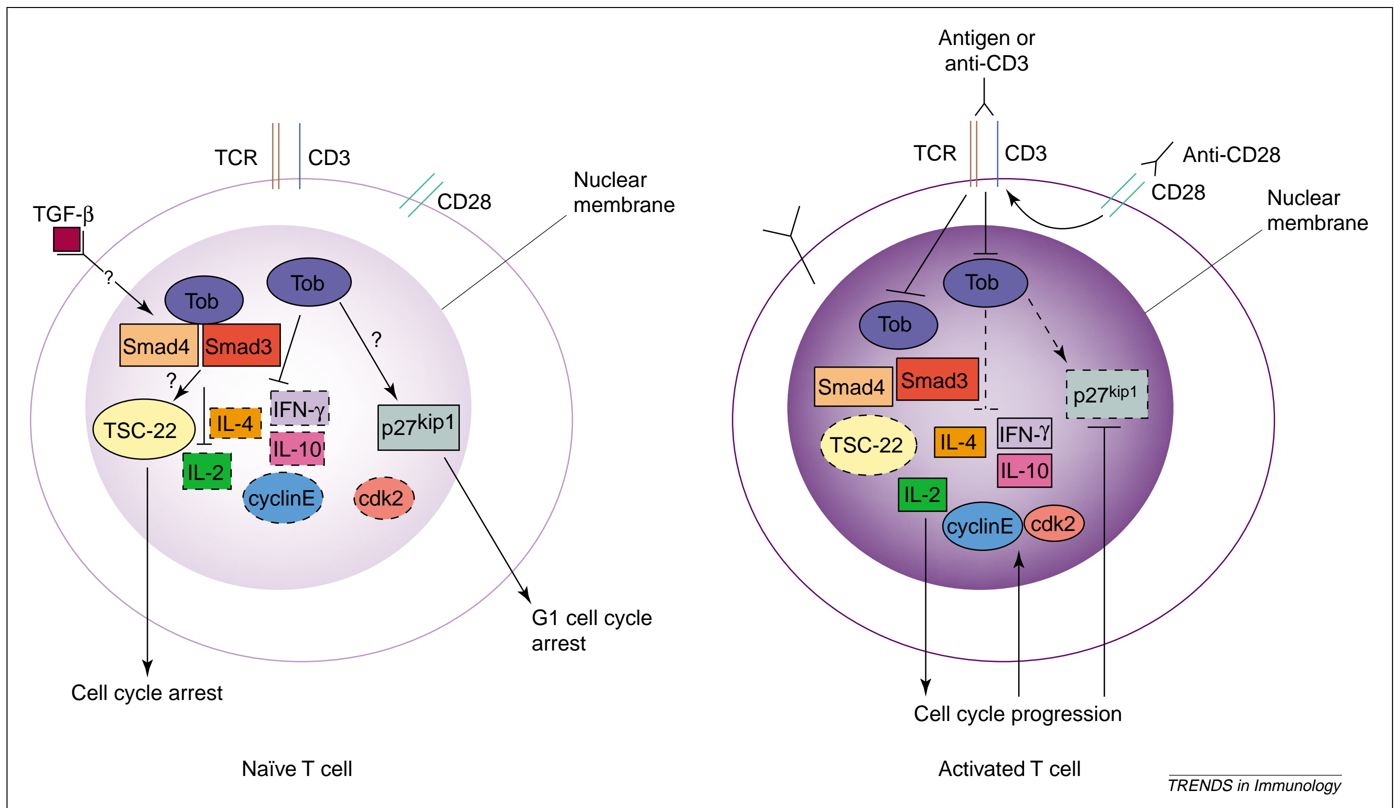

Fig. 2. Tob suppresses T-cell proliferation by antagonizing cytokine production. Interaction between Smads and Tob could be responsible for suppressing interleukin-2 (IL-2) production. Expression of Tob seems to inhibit appearance of cyclins A and E and cyclin-dependent kinase-2 (cdk-2). In addition to this, Tob leads to downregulation of $p27^{kip1}$, a cell cycle inhibitor shown to cause G1 arrest. It is possible that these effects on cell cycle regulators occur as a result of reduced cytokine production. TSC-22 [transforming growth factor-β (TGF-β)-stimulated clone 22] has been shown to have growth suppressive qualities and to be upregulated by TGF-β. Its expression is down-regulated following activation of both T and B cells. Therefore, Tob–Smad complex formation could affect TSC-22 expression, thereby leading to cell-cycle arrest. Unbroken lines indicate function or expression. Broken lines indicate loss of function or expression. Abbreviations: IFN-γ, interferon-γ; TCR, T-cell receptor.

activation but does not trigger spontaneous proliferation. One might suppose that complete loss of Tob function would yield a stronger phenotype than antisense inhibition; however, spontaneous T-cell activation was not reported in a study of mice with a disrupted *Tob* gene [21]. It remains possible that Tob has overlapping functions with other family members in arresting cell-cycle progression in T cells. In any case, it will be important to determine the effects of Tob overexpression on cell size, metabolic rate and expression of activation markers in both normal and leukemic T cells, to ascertain whether or not Tob is a *bona fide* quiescence factor.

Forkhead Box proteins

Members of a large family of mammalian transcription factors possess a Forkhead or winged-helix DNA binding domain. Forkhead Box (FOX) proteins have been divided into subfamilies based on sequence homology. Members of the FOXO (Forkhead Box, class O) family have a broad influence on cell cycle and survival (reviewed in Refs [22,23]; also see Box 1). Indeed, different FOXO target genes have been linked to cell-cycle arrest at different stages (G0, G1, G2 or M), or induction of apoptosis (reviewed in Ref. [23]). Members of the FOXO subfamily include FOXO1, FOXO3 and FOXO4. Before considering a possible role for FOXO proteins in lymphocyte quiescence, we will review their regulation by the phosphoinositide 3-kinase (PI3K) pathway and also the established roles for FOXO proteins in other cellular contexts.

PI3K promotes cell proliferation and survival in many cell types [24]. The lipid products of PI3K mediate the activation of downstream effectors, such as Akt. One group of Akt substrates is the FOXO proteins. The three members of the FOXO family each possess three serine or threonine residues that are present within a consensus phosphorylation motif for Akt: Rxrxx(S/T), in which X is any amino acid [25]. Phosphorylation by Akt (or the related Sgk kinases) leads to functional inactivation of

Box 1. OutFOXed into quiescence

Forkhead box, class O (FOXO) proteins are not the only subclass of Forkhead family transcription factors with probable roles in opposing lymphocyte activation. Mutations in *FOXP3* (Forkhead box, class P, gene 3) are associated with spontaneous activation of $CD4^+$ T cells and lethal autoimmune syndromes in both mice and humans [46,47]. Recently, high expression of *FOXP3* was shown to be a hallmark of the $CD4^+CD25^+$ regulatory T (Tr)-cell subset [48–50]. Moreover, Tr cells are absent from mice lacking functional FOXP3 [49,50]. FOXP3 over-expression could convert naïve T cells to a regulatory phenotype [48–50]. As an essential transcription factor for regulatory T-cell differentiation, FOXP3 can thus be considered a 'non-cell-autonomous' quiescence factor involved in maintaining peripheral tolerance.

FOXO proteins through their release from DNA, binding to 14-3-3 proteins [26] and sequestration in the cytoplasm. Mutation of the three Akt or Sgk phosphorylation sites to alanine ('A3 mutants') results in the FOXO proteins becoming PI3K-independent [27]. The pathway leading from PI3K to Akt to the inactivation of FOXO factors appears to represent an evolutionarily conserved mechanism of regulating metabolic output. In *C. elegans*, nutrient restriction triggers entry into the dauer stage, in which metabolic activity is limited. Dauer entry requires the FOXO ortholog *DAF-16* and is opposed by PI3K and Akt (reviewed in Refs [22,23]).

Forced expression of FOXO proteins causes growth arrest and/or apoptosis in a variety of cell lines (reviewed in Refs [22,23]). Expression of PI3K-independent A3 variants prevents rescue by growth factors, illustrating the central role of PI3K signaling in FOXO inactivation. Transient transfection of FOXO3(A3) caused apoptosis in cerebellar granule cells, CCL39 fibroblasts, or Jurkat T cells [26]. Expression of FOXO4(A3) in A14 fibroblasts caused cell-cycle arrest in G0 or G1 [27]. In the IL-3-dependent BaF3 cell line, inducible activation of an estrogen receptor–FOXO fusion protein [FOXO3(A3)–ER] induced arrest in G0 or G1 and an increase in apoptosis [28]. Similar findings were recently reported in myeloid cells transformed by the human oncogene *Bcr-Abl* [29]. *p27^{kip1}* is a direct FOXO target gene, and in most systems has a role in FOXO-mediated cell-cycle arrest, as well as apoptosis. Fas ligand (FasL) and Bim are other FOXO target genes involved in apoptosis.

A careful analysis by Kops and coworkers demonstrated that, in many cell types, cell cycle arrest induced by FOXO proteins involves reversible exit into G0 rather than G1 arrest [30]. Various lines of evidence were presented to show that *p130* is a direct FOXO target gene required for induction of quiescence (Fig. 3). p130 is a member of the retinoblastoma (Rb) family of 'pocket' proteins that inhibit cell-cycle progression through binding or interference with E2F transcription factors. Mitogenic stimuli trigger increased phosphorylation of pocket proteins and subsequent release of E2F factors that promote proliferation. One of the hallmarks of quiescent cells is a high level of hypophosphorylated p130 and the presence of p130–E2F complexes [30].

Collectively, studies of FOXO function suggest that this family of transcription factors can promote either quiescence or apoptosis, depending on the cellular context. It has been proposed that FOXO-mediated p27^{kip1} upregulation causes the initial cell-cycle arrest, regardless of cell type [30]. In non-hematopoietic cells, induction of p130 predominates and triggers cell-cycle exit into G0. In hematopoietic cells, FOXO proteins cause the upregulation of Bim and/or FasL and apoptosis is favored. Most of the evidence for this model comes from studies of growth-factor-dependent cell lines, rather than primary lymphocytes. For

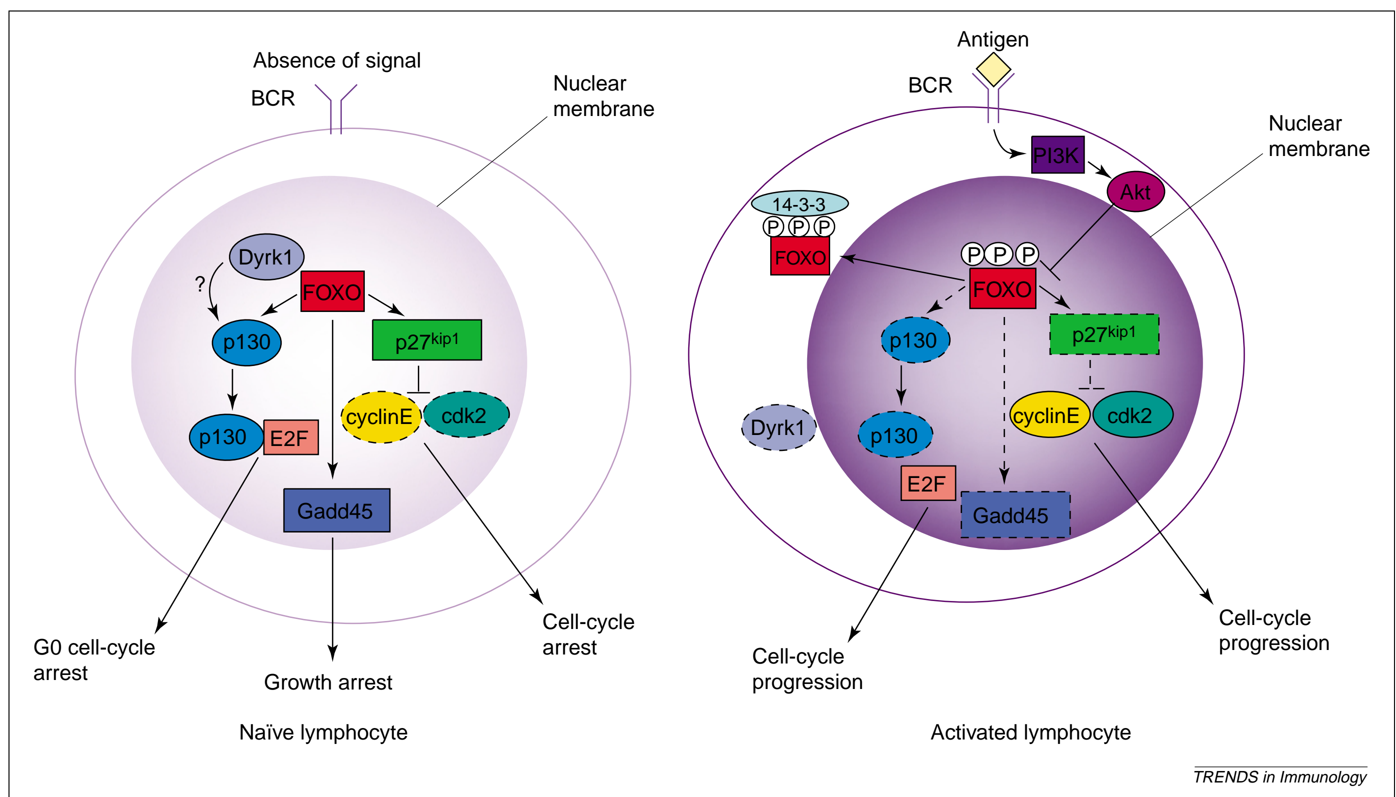

Fig. 3. Forkhead box, class O (FOXO) proteins might promote quiescence in lymphocytes. The FOXO subfamily of forkhead transcription factors influences both cell cycle and survival. They upregulate the expression of cell cycle inhibitors, such as p27^{kip1}, Gadd45 (growth arrest and DNA damage-induced gene 45) and p130. p130 binds E2F transcription factors and thereby inhibits their function, which in turn leads to G0 arrest. The inactivation of FOXO proteins leads to the downregulation of inhibitory proteins and therefore leads to cell-cycle progression. p130 expression is downregulated in activated B cells in a phosphoinositide 3-kinase (PI3K) dependent manner, supporting a role for FOXO proteins in this pathway. Dyrk1, whose expression is downregulated in activated T cells, interacts with FOXO and enhances its transcriptional activity. A role for Dyrk1 in B cells is speculative. Unbroken lines indicate function or expression. Broken lines indicate loss of function or expression. Abbreviation: BCR, B-cell receptor.

example, expression of the FOXO3(A3) variant in IL-2-dependent cytotoxic T-lymphocyte line-2 (CTLL-2) cells upregulates p27kip, Bim and FasL and causes apoptosis [31]. Could FOXO proteins also have a role in quiescence of naïve T or B cells?

This possibility is consistent with the central role of PI3K in activation of naïve T and B cells [32]. However, PI3K signaling has many outputs and the importance of FOXO blockade for lymphocyte activation has yet to be determined. Treatment of human peripheral-blood T cells with anti-CD3 and IL-2 leads to the appearance of phosphorylated FOXO1 within 24 h [31]. FOXO1 is also expressed in murine splenic B cells and its phosphorylation on consensus Akt sites is increased within 5 min of BCR crosslinking in a PI3K-dependent manner (I. Yusuf *et al.* unpublished). Measurement of mRNA levels for ~11 000 murine genes by microarray showed that BCR stimulation of splenic B cells causes rapid, PI3K-dependent downregulation of 16 genes, consistent with possible regulation by FOXO transcription factors [17]. One of these genes is *p130*, a result we have confirmed by RT–PCR (I. Yusuf *et al.* unpublished). As reported previously for the human *p130* gene, consensus FOXO binding sites are present in the regulatory regions of the murine p130 gene. E2F proteins in quiescent B cells are complexed with p130. This complex dissociates during G_1, following B-cell stimulation with either anti-IgM or anti-CD40 [33]. Together, these observations suggest that FOXO proteins might contribute to B-cell quiescence by increasing the expression of p130. Activation of the PI3K pathway through mitogenic ligands would promote cell-cycle entry, in part by inactivating FOXO proteins and reducing p130 expression (Fig. 3). Direct evidence that FOXO proteins regulate lymphocyte quiescence will require genetic strategies to diminish or enhance FOXO function in primary cells.

Other possible quiescence factors

Evidence has accumulated that lymphocyte quiescence is actively maintained by transcription factors, such as LKLF, therefore, there is increasing interest in other factors that might promote the quiescent state. Some candidates have been suggested by global gene expression analyses. In addition to *LKLF*, *GKLF* and *BKLF*, other candidate genes highly expressed in resting T cells and downregulated in activated cells included *TSC-22* (TGF-β-stimulated clone 22) and *Dyrk1* [2].

TSC-22 is upregulated by anti-cancer drugs [34] and has recently been shown to be downregulated in a panel of human brain tumors, suggesting a role in growth suppression and identifying it as a potential tumor-suppressor gene [35]. Antisense inhibition of *TSC-22* expression promoted growth of cancer cells rendered quiescent by a chemotherapeutic drug [34]. Of note, *TSC-22* was originally identified as a gene upregulated by TGF-β, a cytokine with established immunosuppressive properties [36]. Thus, *TSC-22* expression in lymphocytes might be affected by Smad–Tob complexes acting downstream of TGF-β or related ligands (Fig. 2). *TSC-22* expression was also downregulated in a B-cell microarray study [3].

Dyrk1 is a member of a nuclear kinase family proposed to be involved in growth control [37]. Interestingly, Dyrk1 was recently shown to interact with FOXO1 and to co-activate FOXO1-dependent gene expression in a rat hepatoma cell line [38]. It is possible that Dyrk1 enhances FOXO-dependent transcription of p130 and other target genes in resting lymphocytes (Fig. 3).

In B cells, two downregulated genes of particular interest are *Gadd45* and *GILZ* (glucocorticoid-induced leucine zipper) [3]. *Gadd45* is repressed by Myc (Fig. 1) and is induced by FOXO proteins [39] (Fig. 3) and its overexpression in fibroblasts leads to cell cycle arrest [40]. GILZ, which shows homology to TSC-22, binds to both NF-κB and AP-1 (activating protein-1) transcription factor components and inhibits their function [41,42]. Specifically, coexpression of GILZ can inhibit activity of reporter genes driven by NF-κB, AP-1 or the full IL-2 promoter. In this regard, GILZ appears to function like Tob, inhibiting the production of growth factors. GILZ also inhibits activity of the FasL promoter [42]; this finding helps to explain the previous observation that GILZ can protect T cells from anti-CD3-mediated apoptosis [43]. Thus, the relative resistance of quiescent cells to apoptosis could be mediated by the same factors that inhibit the production of cytokines.

Conclusions

Quiescence factors have begun to gain recognition for their broad influence on cell fate. The study of lymphocyte quiescence, although still in its infancy, has already demonstrated that a single transcription factor (e.g. LKLF) is both required for the maintenance of T-cell homeostasis and is sufficient to drive a leukemic T cell into quiescence. Mutations in quiescence factors, such as LKLF, or their targets, could lead, therefore, to the development of lymphoid malignancies.

To date, LKLF is the only transcription factor shown not only to block activation but also to enforce quiescent phenotypes, such as reduced cell size and metabolism. Other factors discussed in this Review (Tob, GILZ) appear to inhibit lymphocyte activation by interfering with cytokine gene induction but have not yet been shown to influence the establishment of a quiescent state. FOXO proteins are attractive candidates for promoting quiescence owing to their known activation of *p130* expression. In addition, FOXO family members have an evolutionary conserved role in limiting metabolism because the *C. elegans* FOXO homolog *DAF-16* is required for entry into dauer arrest (reviewed in Ref. [22]). PI3K activation by CD28 co-stimulation in T cells is crucial for increased metabolic activity that accompanies the transition from quiescence into cell cycle [44]. It is worth investigating whether FOXO transcriptional targets in resting T cells include genes that limit metabolism, as recently documented in *C. elegans* [45].

The available data suggest a model in which a limited number of transcription factors act as master regulators that direct intermediate 'cascades' of transcription factors to influence crucial cell-cycle regulatory proteins. One obstacle to testing the roles of candidate genes in lymphocyte quiescence is the difficulty in manipulating

gene expression in primary resting T and B cells. Transgenic and gene knockout approaches, along with novel technologies, such as protein transduction and morpholinos [19], as well as lentiviral vectors, will provide the tools to help decipher regulatory networks that program quiescence.

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