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The Role of Forkhead Box 1 (FOXO1) in the Immune System: Dendritic Cells, T Cells, B Cells, and Hematopoietic Stem Cells

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Abstract

Forkhead box-O (FOXO) transcription factors have a fundamental role in the development and differentiation of immune cells. FOXO1 and FOXO3 are FOXO members that are structurally similar and bind to the same conserved consensus DNA sequences to induce transcription. FOXO1 has been studied in detail in the activation of dendritic cells (DCs), where it plays an important role through the regulation of target genes such as ICAM-1, CCR7, and the integrin $\alpha\text{v}\beta 3$. FOXO1 is activated by bacteria challenge in DCs and promotes DC bacterial phagocytosis, migration, homing to lymph nodes, DC stimulation of CD4⁺ T cells and resting B cells, and antibody production. Deletion of FOXO1 in DCs enhances susceptibility to bacteria-induced periodontal disease. FOXO1 and FOXO3 maintain naive T cell quiescence and survival. FOXO1 and FOXO3 enhance the formation of regulatory T cells and inhibit the formation of T-helper 1 (Th1) and Th17 cells. FOXO1 promotes differentiation, proliferation, survival, immunoglobulin gene rearrangement, and class switching in B cells, but FOXO3 has little effect. Both FOXO1 and FOXO3 are important in the maintenance of hematopoietic stem cells by protecting them from oxidative stress. This review examines FOXO1/FOXO3 in the adaptive immune response, key target genes, and FOXO inhibition by the phosphoinositide 3-kinase/AKT pathway.

Keywords

protein kinase B (AKT); antibody; acquired immunity; forkhead; inflammation; lymphocyte

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I. INTRODUCTION

The first forkheadbox (FOX) transcription factor was identified in mammalian cells by homology with the forkhead gene found in *Drosophila*¹ and subsequently cloned and characterized in humans.² Since then, many other transcription factors sharing the forkhead DNA-binding domain of approximately 100 amino acids have been identified. The initial discovery was made in the fruit fly and homologs have been found in species ranging from fungi to mammalian cells, suggesting a critical evolutionary-conserved biological role for these proteins.³ This large family of transcription factors has been grouped into subfamilies according to their structural characteristics and are currently the largest family of transcription factors in humans.⁴ The forkhead box “O” subfamily has four members in humans, of which three (FOXO1, FOXO3, and FOXO4) have a high degree of sequence homology⁵ and one (FOXO6) is more distantly related with a more restricted expression and distinct regulatory mechanisms.⁶ FOXO proteins regulate cell survival, cell cycle, and embryonic pattern formation,^{7,8} which are closely related to their involvement in cancer.⁹ *In vitro* studies suggest a similar biological activity for FOXO1, FOXO3, and FOXO4 and, in some cases, the regulation of similar target genes by binding to the same conserved DNA sequence. However, disruption of FOXO1 in mice is embryonically lethal at day 10.5, whereas animals lacking either FOXO3 or FOXO4 were viable and grossly similar to wild-type littermates. The primary phenotypes observed in FOXO3-deficient mice are infertility from abnormal ovarian follicular development,¹⁰ abnormal proliferation of lymphatic cells, increased inflammation,¹¹ and a reduced neural stem cell pool.¹² Deletion of FOXO4 enhances response to inflammatory stimuli¹³ and deletion of FOXO6 results in impaired memory and learning.¹⁴ Therefore, the biological functions of FOXOs are complex and sometimes overlapping, but are not completely redundant.

FOXOs may act as transcriptional factors by inducing the expression of target genes with FOXO response elements. FOXO activation is complex, involving not only transcriptional activation, but also various post-transcriptional and post-translational mechanisms, including miRNA-mediated repression;¹⁵ acetylation, phosphorylation, ubiquitination, methylation, and glycosylation;¹⁶ protein-protein interactions; and cytoplasmic-nuclear shuttling.¹⁷ Alterations in FOXO1 affect its nuclear import (activation) or export (inactivation) and DNA-binding activity. FOXOs have four functional motifs, which include a forkhead DNA-binding domain and domains that control nuclear localization, nuclear export, and transactivation. These domains are highly conserved. FOXOs recognize two different consensus DNA-binding sequences: a Daf-16 binding element (5'-GTAAA(T/C)AA) and an insulin-response element (5'-(C/A)(A/C)AAA(C/T)AA). The core DNA sequence 5'-(A/C)AA(C/T)A is recognized by all FOXO family members. Kinases and acetylases modulate the nuclear localization and nuclear export to control shuttling of FOXOs. The chaperone protein 14-3-3 binds to FOXOs in the nucleus, exports them,¹⁸ and in turn blocks them from returning to the nucleus.¹⁹

FOXOs are phosphorylated by several kinases to modulate FOXO subcellular location, DNA-binding, and transcriptional activity.^{20,21} A major negative regulator of FOXOs is the phosphoinositide 3-kinase (PI3K) pathway. PI3K activation induces the recruitment of the kinases AKT and serum/glucocorticoid regulated kinase 1 (SGK1) to the cell membrane,

where each is activated by phosphorylation. AKT and SGK1 phosphorylate FOXO transcription factors directly on three different sites to inactivate FOXOs. Phosphorylation of FOXO1 or FOXO3 by AKT or SGK1 decreases FOXO DNA-binding affinity to consensus response elements and also increases their association with 14–3–3 proteins, which leads to inactivation by transport out of the nucleus. In contrast, phosphorylation of FOXOs at different amino acid residues by other kinases can have the opposite effect, demonstrating the complexity of FOXO activation. This alternative phosphorylation can increase nuclear localization to enhance FOXO activity. Kinases that stimulate FOXO activity include c-Jun N-terminal kinase (JNK), p38, 5' AMP-activated protein kinase (AMPK), and cyclin-dependent kinase 1. Similar to phosphorylation, acetylation has been shown to both promote and decrease FOXO transcriptional activity and to mediate different biological functions of FOXOs.^{20,21} The deacetylation of FOXO generally increases FOXO activity, whereas acetylation reduces it. For example, silent information regulator 1 (Sirt-1) and Sirt-2 belong to the sirtuin family of deacetylases and lead to FOXO deacetylation, increasing their binding to DNA.²² Ubiquitination also regulates FOXO proteins. FOXO undergoes degradation through polyubiquitination, which functionally deactivates FOXOs. However, monoubiquitination of FOXOs can increase nuclear localization, effectively enhancing FOXO activity.²³ FOXOs also interact with β -catenin. When FOXOs bind to β -catenin in osteoblasts, β -catenin is not available to bind to T cell factor, thus diminishing T cell factor activity.²⁴ In this case, FOXOs act as a transcriptional repressor by ultimately reducing T cell factor activity. In CD8+ T cells, reduced levels of FOXO1 lead to increased stimulatory T cell factor-1 through a similar mechanism.²⁵

FOXOs have a fundamental role in the maintenance of organism homeostasis and adaptation to environmental changes,²⁶ which includes the homeostasis and development of immune-relevant cells in higher vertebrates.²⁷ More recently, the involvement of FOXO1 and FOXO3 in diverse functional aspects of the innate and adaptive immune response such as dendritic cell (DC) activity,^{28,29} CD8 T cell response to chronic viral infections,³⁰ macrophage activation in parasitic³¹ and bacterial infections by Gram-negative lipopolysaccharide (LPS),^{32,33} and antibody class switching by B cells have begun to be explored.³⁴ This review focuses on the role of FOXO1 and FOXO3 in the biology of DCs, B and T lymphocytes, and hematopoietic stem cells (HSCs).

II. DCs

DCs express high levels of major histocompatibility complex class II (MHCII), with cytoplasmic projections similar to the dendrites of neurons.³⁵ Two major DC subsets can be identified in secondary lymphoid tissues: plasmacytoid DCs (pDCs) and myeloid DCs (mDCs).³⁶ pDCs are recruited to sites of inflammation and are present in lymph nodes,³⁷ but are not considered to be potent antigen-presenting cells (APCs).³⁸ mDCs are a heterogeneous population of cells that are associated with activation of adaptive immunity through antigen presentation.³⁸

As specialized APCs, DCs connect the innate and adaptive arms of the immune response.³⁸ The biological activation and function of DCs depends on pattern recognition receptors (PRRs) such as C-type lectin receptors, toll-like receptors (TLRs) and Nod-like receptors, as

well as by cytokine and chemokine receptors.³⁹ DCs can respond to a variety of external antigens of microbial and non-microbial origin (bacterial, viral, fungal, protozoa, allergens, etc.) as well as damage-associated molecule patterns. Ultimately, the combination of PRRs and other stimulated receptors determines subsequent activation of DCs.³⁹

DCs are phagocytic in their immature state⁴⁰ and acquire enhanced antigen-presenting and migration capacities upon maturation.^{41,42} Activated DCs migrate to lymph nodes for effective antigen presentation by up-regulating the expression of MHC and co-stimulatory molecules (CD80 and CD86).⁴³ DCs from the peripheral circulation and DCs residing in non-lymphoid tissues (e.g., Langerhans cells in the skin) are guided to lymph-node-produced CCL19/ CCL21, which stimulate the chemokine receptor CCR7 on DCs. Activated DCs produce various cytokines, including interleukin (IL)-1, IL-6, IL-10, IL-12, IL-23, IL-27, and tumor necrosis factor- α (TNF α),⁴⁴ which will affect the activation and biological activity of other innate and adaptive immune cells. However, immature (i.e., non-activated) DCs may exert an inhibitory effect.⁴⁵ Cytokines such as TNF α and type I interferons (IFNs) and other PRRs induce multiple signaling pathways (e.g., mitogen-activated protein kinase [MAPK], MyD88, Raf/ PI3K, mammalian target of rapamycin [mTOR], and nuclear factor kappa light-chain enhancer of activated B cells [NF- κ B]) that induce DC activation.⁴⁶ PI3K can be activated by specific stimuli to induce AKT and negatively regulate FOXOs.⁴⁷

mTOR and AKT play a role in controlling the inflammatory response in DCs through regulation of FOXO1. Deletion of Rictor, a key component in mTOR signaling, causes a hyperinflammatory response in DCs. The link between mTOR and FOXO1 is mediated by AKT.⁴⁷ FOXO1 is phosphorylated by AKT, which causes FOXO1 to relocate to the cytoplasm. When FOXO1 is overexpressed *in vitro*, DCs produce high levels of IL-12, IL-6, and TNF α and, when FOXO1 is deleted, DCs have reduced capacity to produce inflammatory cytokines.²⁹ Therefore, FOXO1 promotes induction of inflammatory cytokines in DCs; in contrast, activation of mTOR and AKT deactivate FOXO1 to prevent a hyperinflammatory response.⁴⁷

Bacterial infection by anaerobic Gram-negative periodontal pathogens such as *Porphyromonas gin-givalis* or LPS stimulates FOXO1 nuclear localization through the MAPK pathway.²⁸ FOXO1 induces transcriptional activity and stimulates expression of adhesion molecule ICAM-1, integrins α v and β 3, as well as CCR7 and matrix metalloproteinase-2, which are needed for DC activity.^{28,29} By regulating these downstream target genes, FOXO1 promotes DC migration to lymph nodes and re-circulation to infected non-lymphoid tissue.^{28,29} FOXO1 regulation of CCR7 and ICAM-1 is particularly important in homing to lymph nodes because reduced DC homing in *FOXO1* deleted DC is rescued by overexpression of ICAM1 and CCR7.³⁰ FOXO1 is also needed for DC stimulation of T and B lymphocytes, which can be linked to reduced capacity of DCs to bind to lymphocytes when FOXO1 is absent. Bacteria-induced stimulation of IFN- γ and IL-13 by CD4⁺ T cells from lymph nodes is reduced by 50–65% when *FOXO1* is specifically ablated in DCs and FOXO1 ablation reduces the presentation of antigens *in vitro*.²⁸ There is reduced production of BAFF and APRIL in DCs lacking FOXO1, which accounts for their reduced antibody production and capacity to stimulate B cell proliferation.²⁸

Bacterial inoculation stimulates the migration of DCs. When mucosal tissue is inoculated with bacteria, deletion of FOXO1 reduces the number of DCs that migrate to the infected tissue.²⁹ FOXO1 deletion down-regulates genes that play an important role in DC migration including integrin α_v , integrin β_3 , and matrix metalloproteinase-2.²⁹ DC deletion of *FOXO1* decreases the formation of plasma cells in the lymph nodes,²⁹ consistent with reports that FOXO1 is needed for DC activation of B cells.²⁸ The ultimate effect of reduced FOXO1 in DCs is to attenuate the capacity of mice to generate antibodies in response to bacterial infection by *P. gingivalis*. The functional significance of FOXO1 activation in DCs is shown by increased susceptibility to bacterial infection in mice that lack FOXO1 in DCs, leading to greater inflammation and periodontal bone loss.²⁹ Therefore, FOXO1 coordinates DC activity by regulating the expression of downstream target genes that are needed for DCs to stimulate T and B lymphocytes and generate an antibody defense to bacteria,^{28,29} as shown in Fig. 1.

III. T CELLS

Naive T cells are activated by “professional” APCs and can differentiate into distinct phenotypical subsets such as T helper-1 (Th1), Th2, Th17, Th9, or regulatory T cells (Tregs). FOXO1 plays an important role in the adaptive immune response by promoting the formation of Tregs, the development of the B lymphocytes, and maintaining a pool of

HSCs.^{48,49} FOXO proteins regulate several aspects of lymphocyte function, including lymphocyte development, cytokine expression, gene recombination, and homing.⁵⁰ FOXO1 is needed for the survival of naive T cells and homing to secondary lymphoid organs⁵¹ by regulating the expression of *L-selectin* and sphingosine-1-phosphate receptor 1 (*S1pr1*), which are important for lymphocyte transit to and from lymphatic tissue. Activation of STAT3 inhibits T cell proliferation by up-regulating FOXO1 and FOXO3, which maintain T cells in a quiescent state and enhances their survival.⁵⁰ FOXO1 also enhances T cell survival by regulation of IL-7 receptor α (IL-7 α), a subunit of the survival receptor for naive T cells. Interestingly, although FOXO1 is required for naive T cell survival, FOXO3 appears to have the opposite effect, increasing apoptosis. The basis for this differential response of FOXO1 and FOXO3 on T cell survival is not known. FOXO1 and FOXO3 share the ability to inhibit T cell activation, leading to a decrease in calcineurin/nuclear factor and less hypertrophic cardiomyopathy.⁵²

A. Tregs

Tregs are formed in the thymus and play a critical role in deactivating or suppressing the immune response by the reducing activity of effector T cells (Th1, Th17, etc.). The differentiation and function of Tregs is impaired by deletion of *FOXO1* and the addition of *FOXO3* makes this impairment more severe. Tregs are controlled by the transcription factor Foxp3. Mice with T cell-specific combined deletion of *FOXO1* and *FOXO3* have many of the same defects as mice with *Foxp3* deletion, including impaired Treg formation and deficient *Foxp3* expression. FOXO1 stimulates the formation of Tregs by modulating the expression of several different genes. FOXO1 and FOXO3 bind to the promoter region of *Foxp3* and cytotoxic T lymphocyte antigen 4 (*Ctla4*) and regulate directly *Foxp3* and *Ctla4*

promoter activity as well as several other genes needed for development of Tregs.⁵³ Interestingly, FOXO1 appears to regulate a different set of genes compared with Foxp3.⁵⁴ Therefore, Foxp3 alone is not sufficient to induce Treg differentiation and requires the participation of FOXO1.

FOXO1 and TGF- β are critical for inducing differentiation and proliferation of Treg cells.⁵² Mice with *FOXO1*^{-/-} naive T cells have drastically reduced levels of Treg cells and the Tregs that are produced have reduced function and viability.^{55,56} Moreover, in the absence of FOXO1, TGF- β expression leads to the formation of Th1 cells. This indicates that, in addition to driving Treg formation, FOXO1 and FOXO3 prevent naive T cells from acquiring T cell effector function.⁵⁷

Tregs have high levels of FOXO1 and reduced activity of PI3K/AKT. Reduced AKT activity is required to increase FOXO1 activation and enhances Treg formation. The diminished AKT activity may be mediated by PTEN.⁵⁸ Interestingly, inflammatory conditions that activate the PI3K/AKT pathway repress Treg differentiation and function.⁵⁹ Another critical factor in forming Tregs is the inhibition T-box expressed in T cells (Tbet). Tbet directs naive T cells away from Treg differentiation and is induced by IFN- γ . FOXO1 and TGF- β work together to inhibit IFN- γ and Tbet expression to enhance the formation of Tregs.⁶⁰ Without FOXO1, TGF- β is less effective. This relationship is significant because the absence of *FOXO1 results in greater* T cell expression of IFN- γ , which in turn contributes to autoimmunity *in vivo*. *This phenomenon is further worsened by the combined deletion of both FOXO1 and FOXO3.*⁶⁰ Constitutively active FOXO1 and STAT5 (with high TGF- β and low IL-6) were shown to rescue this autoimmune phenotype.⁴⁸ However, it is important to note that, in diabetic conditions, the relationship between FOXO1 and TGF- β 1 changes. FOXO1 in a hyperglycemic environment fails to bind to the TGF- β 1 promoter and fails to upregulate TGF- β 1 transcription.⁶¹ The consequence of having less TGF- β 1 is growth-factor-deficient healing, which can be rescued in FOXO1-deficient mice by application of exogenous TGF- β 1.⁶¹ In contrast, under normal glucose levels, FOXO1 binds efficiently to the TGF- β 1 promoter and upregulates TGF- β 1 transcription and expression to promote healing. This change in FOXO1 behavior and the ability to induce TGF- β 1 depends on glucose levels.

FOXO1 is also needed for Treg function. For example, an important downstream target of FOXO1 in Tregs is CCR7.⁵⁴ As was noted for DCs, FOXO1-induced expression of CCR7 is important in Treg homing.⁵⁴ Interestingly, mice with a conditional deletion of *FOXO1*^{-/-} only in Tregs have reduced Treg function and display similar pathology to mice with low Treg populations *in vivo*. These mice have enlarged lymph nodes and a swollen spleen associated with an increase in the number of T cells.⁵³ Figure 2 describes the role of FOXO1 and FOXO3 in Treg cell function.

B. Th1

Th cells develop into effector T cells, one of which is Th1. Th1 cells promote inflammation, amplify the innate immune response, and are important in the host response to intracellular pathogens such as viruses. IL-12 signaling stimulates IFN- γ expression to induce T-bet, the master regulator of Th1 cell differentiation. As mentioned above, FOXO1 inhibits T-bet and

drives naive T cells to form Tregs. Therefore, the net effect of active FOXO1 is to inhibit the differentiation of Th1 cells. To form Th1 cells, it is necessary to reduce FOXO1 activity, which is largely accomplished by stimulating AKT. Antigen presentation to the T cell receptor (TCR) activates PI3K, and subsequently, AKT. This leads to FOXO1 phosphorylation and its subsequent inactivation.⁵² IL-12 receptor signaling extends and maintains AKT phosphorylation. In addition, Th1 cells produce IL-2, which further activates the AKT pathway to inhibit FOXO1.⁶² Therefore, Th1 cell differentiation and proliferation requires the inhibition of FOXO1 (which is active in basal naive T cells). There are multiple signaling pathways that lead to activation of AKT and inhibition of FOXO1. Too much inhibition of FOXO1 via the AKT/PI3K and other pathways can lead to preferential differentiation to Th1 cells, resulting in a higher likelihood of autoimmunity.⁵²

C. Th17

Th17 cells are important in the host response at mucosal surfaces.⁶³ Th17 cells mediate the recruitment of neutrophils and macrophages to infected tissues. They are characterized by production of IL-17 and acquire effector function in the thymus. Like Th1 cells, there is polarization between Tregs and Th17 cells. Differentiation of Th17 cells is controlled by a master regulator of Th17 cell differentiation, the transcription factor retinoic acid receptor-related orphan receptor-gamma-t ($ROR\gamma t$). Th17 cells are induced by IL-23 and IL-1 β , which stimulate signal transducer and activator of transcription 3 (Stat3). FOXO1 activity inhibits the formation of Th17 cells and suppresses the expression of IL-17A. FOXO1 inhibits IL-17A and IL-23 receptor expression in part by inhibiting the transcriptional activity of $ROR\gamma t$ ⁶⁴ by forming a complex with it. Therefore, FOXO1 acts a repressor of $ROR\gamma t$ to inhibit Th17 differentiation.⁶⁴ Because of the inhibitory effect of FOXO1 on the formation of Th17 cells, antigen activation of the TCR is needed to reduce FOXO1 activity and permit Th17 cell differentiation. One of the key effector molecules in Th17 cells that inhibits FOXO1 activation is SGK1.⁶⁵ Similar to AKT, SGK1 phosphorylates FOXO1 and sequesters it to the cytoplasm for degradation.⁶⁵ In addition, FOXO1 suppression via the PI3K/AKT pathway is important in the formation of Th17 cells.⁶⁴ Mice with a *FOXO1*^{-/-} ablation in their T cells have increased numbers of Th17 cells, establishing FOXO1's ability to limit differentiation to Th17 lymphocytes *in vivo*.⁶⁴ Figure 3 describes the role of FOXO1 inhibition in the formation of Th17 cells.

D. Memory T Cells

Memory T cells are effector cells that have been exposed previously to a specific antigen and are referred to as “antigen-experienced T cells,” which can produce a more rapid and stronger immune response. Memory T cells are produced from CD8 and CD4 effector T cells through a differentiation pathway that requires precise modification of gene expression in the effector cells. When FOXO1 is inhibited by the PI3K/AKT pathway, effector T cell status is preserved at the expense of memory T cell development.⁶⁶ FOXO1 becomes active in the formation of memory T cells and reduced FOXO1 can inhibit the formation of memory T cells.⁵⁵ In contrast, activation of FOXO1 can inhibit effector T cell function and favor memory T cell formation based in part on its ability to repress Tbet signaling. FOXO1 is also critical for proper functioning and clonal expansion of the memory T cell upon second (repeated) contact with an antigen. In support of this, *FOXO1* deletion reduces the

number of memory T cells capable of expanding and differentiating. In contrast, FOXO3 activation enhances the maintenance of memory T cells. Figure 4 describes the role of FOXO1 in memory T cell function.

IV. B CELLS

B cells produce antibodies, present antigen, and affect inflammation by generating cytokines. In mammals, B cells are produced and mature in the bone marrow and circulate in the blood and secondary lymphatic organs. B cells that have been stimulated with an antigen undergo class switching and differentiate into plasma cells to produce high-affinity antibodies. B cells express B cell receptors (BCRs) on their cell membranes that bind specific antigens. B and T cells are activated via similar mechanisms: both cells react to antigen binding to the BCR or TCR, respectively, which leads to the activation of a complex signaling cascade.⁶⁷ Both BCRs and TCRs initiate signaling that contribute to PI3K activation. PI3K, in turn, activates AKT and inhibits FOXO1 and FOXO3 activity. Mice with a mutation in PI3K that prevents activation of AKT display similar immunodeficiency to CD19^{-/-} mice in that both have reduced capacity to produce antibodies in response to antigen challenge.⁶⁸ LPS-challenged primary B cells with constitutively active Foxo1 display decreased proliferation and increased cell death.⁶⁸

FOXO1 is upregulated at the early pro-B cell stage.^{69,70} Deletion of *FOXO1* impairs several stages of B cell development due to FOXO1 regulation of key target genes, particularly IL-17 receptor alpha (*Il7ra*), recombination activating gene 1 (*RAG1*), and *RAG2*, *L-selectin*, *Aicda*, and early B cell factor 1 (*EBF1*).^{69,70} These findings establish FOXO1 as a critical member of the transcription factor network directing early B cell development and peripheral immune function. Interestingly, deletion of *FOXO3* in B cell progenitors does not have a major effect on B cell differentiation or function.

Genetic deletion of *FOXO1* in lymphoid progenitor cells prevents them from forming mature B cells. FOXO1 activation begins during the pre-pro-B cell phase.⁷¹ A key event in B cell differentiation is the expression of EBF1. EBF1 participates in maintaining the B cell lineage and antagonizing differentiation into other lymphocyte subsets by modulating gene expression. EBF1 expression is stimulated by the binding of transcription factors E2A and FOXO1 to their respective consensus response elements to induce EBF1 promoter activity.⁷¹ The binding of both E2A and FOXO1 to the *EBF1* promoter is required because one without the other fails to induce B cell differentiation. Furthermore, E2A and EBF1 both bind to their respective loci on the *FOXO1* promoter to enhance FOXO1 expression, representing feedforward amplification.

FOXO1 regulates several other important genes needed for B cell function. FOXO1 induction of IL-7R α prevents apoptosis and maintains B cell viability.^{69,70} FOXO1 drives L-selectin expression, which is needed for normal recirculation of B cells. RAG1 and RAG2 expression are necessary for the rearrangement of the immunoglobulin light chain. Knock-down of FOXO1 reduces RAG expression, whereas overexpression of FOXO1 increases it. Therefore, FOXO1 is critical for Ig gene rearrangement through its regulation of RAG1 and RAG2.⁷¹ In contrast, knock-down or overexpression of FOXO3 does not modulate RAG

levels, which is consistent with reports that FOXO3 does not play an important role in B cell development. Deletion of FOXO1 at later stages of B cell development also has an effect by blocking class-switching recombination. Deletion of FOXO1 in transitional B cells has no effect on the production of antigen-specific IgM, but does block class-switched antibody formation. Blockage of class switching by deletion of FOXO1 is likely due to its dependence on Aicda. Therefore, FOXO1 induces Aicda, which in turn induces class switching.

V. HSCs

HSCs are located in the bone marrow and exist for a lifetime to lead to the production of leukocytes.⁷² The development of leukemia and lymphoma is greatly affected by factors that mediate proliferation and survival of HSCs. Activation of the PI3K/AKT pathway and inhibition of FOXOs play an important role in the development of cancers that originate from HSCs.⁷³ The role of FOXOs in HSCs was investigated in mice with combined lineage-specific deletion of *FOXO1*, *FOXO3*, and *FOXO4*. FOXO4 is related to FOXO1 and FOXO3, but has been less studied than the other two. The combined *FOXO* deletion in HSCs drove these cells from a quiescent state to entry into the cell cycle, indicating that FOXOs inhibit proliferation.⁷⁴ FOXOs accomplish this inhibition in HSC by inducing expression of negative cell-cycle regulators p21 and p27 and reducing positive regulators such as cyclin D2, as has been shown in other cell types.⁷⁵ The long-term effect of deleting *FOXO1/3/4* in HSCs is due to interference with HSC self-renewal due to an increase in reactive oxygen species. FOXOs are protective by mediating expression of genes that have anti-oxidant properties.⁷⁵ *FOXO1/3/4* combined deficient HSCs have reduced levels of anti-oxidants, including the superoxide dismutase genes *Sod1* and *Sod3*.⁷⁴ It is striking that anti-oxidant treatment of mice with lineage-specific deletion of *FOXO1/3/4* in HSCs restores HSC number and function. It also corrects abnormalities in apoptosis and the cell cycle caused by FOXO deletion. Therefore, FOXOs promote quiescence and survival in the HSC compartment that is required for its long-term regenerative potential.⁷⁴

VI. CONCLUSION

FOXO transcription factors, particularly FOXO1 and FOXO3, play important roles in stimulating an adaptive immune response. Through lineage-specific deletion in mice, the role of FOXOs has been elucidated and were found to be involved in the activation and regulation of DCs. Reduced FOXO1 in DCs leads to diminished cytokine production, impaired homing of DCs to lymph nodes, reduced DC stimulation of CD4+ T and B cells, reduced numbers of plasma cells in lymph nodes, and reduced antibody production. In T and B cells, FOXO1 and FOXO3 activation or inhibition is critical for differentiation and function. FOXOs are important in HSC self-renewal in part by maintaining a quiescent state and protecting HSCs from oxidative stress. Even though FOXO1 and FOXO3 bind to similar conserved consensus DNA sequences, their function may or may not overlap. FOXO1 is required for B cell differentiation, immunoglobulin gene rearrangement, and class switching, but FOXO3 is not. In contrast, both FOXO1 and FOXO3 are needed for the maintenance of HSCs. The mechanisms by which FOXOs are regulated are less understood. They are highly regulated by levels of expression; by post-translational modification, which affects nuclear localization and/or DNA-binding activity; and by partnering with specific co-repressors or

co-activators.⁵² Considerable insight into how FOXOs are modulated and their impact on different cell types comes from studies examining diabetes.⁷⁶ For example, in normal wound healing, FOXO1 plays a positive role in promoting keratinocyte behavior to enhance healing, whereas diabetic conditions alter FOXO1-induced gene targets to inhibit healing.^{77,78} Therefore, FOXO behavior may depend on the cell type and specific conditions, which is a cautionary tale in not extrapolating FOXO function from one cell type to another or one condition to another. This suggests that FOXOs are tightly regulated by epigenetic considerations, which is certain to be a highly investigated topic.

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ABBREVIATIONS:

AKT	protein kinase B
DC	dendritic cell
EBF1	early B cell factor 1
FOX	forkhead box
HSC	hematopoietic stem cell
IFN-γ	interferon-gamma
IL	interleukin
JNK	c-Jun N-terminal kinase
MAPK	mitogen-activated protein kinase
mTOR	mammalian target of rapamycin
NF-κB	nuclear factor kappa light-chain enhancer of activated B cells
PI3K	phosphoinositide 3-kinase
RAG	recombination activating gene
RORγt	retinoic acid receptor-related orphan nuclear receptor gamma
SGK1	serum/glucocorticoid regulated kinase 1
Tbet	T-box protein 21
TCR	T cell receptor
TGF-β	transforming growth factor beta
Th1	T helper-1

Th17	T helper-17
TNFα	tumor necrosis factor alpha
Treg	regulatory T cell

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FIG. 1:

FOXO1 regulates activation and function of dendritic cells. (A) PRRs such as TLRs and cytokine receptors activate FOXO1 in DCs. FOXO1 up-regulates the expression of target genes ICAM1, $\alpha_v\beta_3$, APRIL, BAFF, Ccr7, CD80, CD86, IL-6, IL12, IFN- γ , and TNF- α while decreasing IL-10. This leads to increased homing of DCs to the lymph nodes and bacteria-infected tissue. It also increases the immune response: antigen presentation, T and B cell activation, plasma cell numbers, and inflammation. The role of FOXO3 in DCs has not been well studied. (B) Pattern recognition receptors and cytokine receptor stimulation initiates a signaling cascade to activation of FOXO1 that involves the MAPK pathway. Activated FOXO1 can bind to the promoter region of target genes and regulate transcription. AKT is a major downstream target of PI3K that functions as a negative regulator of FOXO1.

Stimulation of mTOR activates AKT to reduce FOXO1 activity and prevent a hyperinflammatory response.

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FIG. 2:

FOXO1 is highly expressed in Tregs. FOXO1 can induce the expression of Foxp3, Ctla4, and CCR7 to increase the formation of Tregs and enhance Treg function. FOXO1 induces a transcriptome that is distinct from that induced by Foxp3. The effect of FOXO1 is to decrease effector Th cell formation, increase Treg differentiation, and increase the number of Tregs.

FIG. 3:

FOXO1/3 interferes with Th17 effector cell formation. Antigen presentation stimulates the TCR and silences FOXO1/3 through PI3K/AKT and SGK1. FOXO1/3 inhibits IL-23R and ROR γ t expression to inhibit Th17 cell differentiation. For Th17 cells to form, it is necessary to block FOXO1/3 activity.

FIG. 4: FOXO1 increases memory T-cell formation. FOXO1 increases expression of CCR7, IL7R, BCL2, Sell, and Tcf7, which leads to increased memory T-cell formation and function. FOXO1 can decrease Tbet, which facilitates memory cell differentiation. FOXO3 plays a role in the maintenance of memory T cells.