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The FOX transcription factors regulate vascular pathology, diabetes and Tregs

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1. ABSTRACT

A small number of upstream master genes in "higher hierarchy" controls the expression of a large number of downstream genes and integrates the signaling pathways underlying the pathogenesis of cardiovascular diseases with or without autoimmune inflammatory mechanisms. In this brief review, we organize our analysis of recent progress in characterization of forkhead (FOX) transcription factor family members in vascular pathology, diabetes and regulatory T cells into the following sections: (1) Overview of the FOX transcription factor superfamily; (2) Vascular pathology of mice deficient in FOX transcription factors; (3) Roles of FOX transcription factors in endothelial cell pathology; (4) Roles of FOX transcription factors in vascular smooth muscle cells; (5) Roles of FOX transcription factors in the pathogenesis of diabetes; and (6) Immune system phenotypes of mice deficient in FOX transcription factors. Advances in these areas suggest that the FOX transcription factor family plays important roles in vascular development and in the pathogenesis of autoimmune inflammatory cardiovascular diseases.

2. INTRODUCTION

The forkhead (FOX) transcription factor family regulates a large number of major regulatory genes that expansively regulate still more signaling pathways, integration points, and pathological processes. Given such a position atop a regulatory hierarchy, it is easy to see why studies of FOX transcription factors have grown exponentially since their discovery. While we cannot propose to detail every known aspect of FOX transcription factors within the scope of a single review, we can relate the roles of

FOX transcription factors in well-worn fields of medical research that could derive beneficial therapeutic strategies.

Atherosclerosis is characterized by focal arterial lesions containing cholesterol, fibrosis, intense immunological activity, inflammatory cell infiltrates and cell death(1). Several risk factors have been identified for the atherogenic process including hyperlipidemia, low density lipoprotein (LDL), cigarette smoking, diabetes, hypertension, obesity(2) and excessive quiescence(3). Wang's laboratory and others have confirmed that hyperhomocysteinemia (HHcy) also acts as an independent risk factor in accelerating atherosclerosis(4-8). In addition, atherosclerosis is positively correlated with the endotoxin load in patients' plasma(9). These risk factors independently or synergistically lead to chronic vascular inflammation, which is an essential requirement for the progression of atherosclerosis in patients(10). Most recently, Yang's laboratory and others have shown that transcription factor Foxp3-controlled CD4+CD25^{high} regulatory T cells(11) suppress vascular inflammation(12, 13), diabetes(14) and atherosclerosis(15)(16). Despite significant advances in elucidating atherosclerotic pathology, atherosclerosis remains as the leading cause of morbidity and mortality in industrialized society. Therefore, continuous improvement of our understanding of the atherogenesis and vascular inflammation initiated and promoted by risk factors will lead to the future development of novel therapeutics for ischemic stroke, myocardial infarction and other cardiovascular diseases.

In understanding the molecular signals underlying vascular inflammation and metabolic stress, the expression profiles of numerous genes in vascular cells in response to inflammation and metabolic stress stimuli have been identified(15, 16). The question remains whether there are a small number of upstream master genes in "higher hierarchy" that controls the expression of a large number of downstream genes and integrates the signaling pathways underlying vascular development and the pathogenesis of cardiovascular diseases. Among these master genes are several transcription factor families, notch signaling components and epigenetic machinery. Recent reviews more specifically detail FOX transcription factors(17), myocardin related transcription factors(18), notch signaling(19), Kruppel-like transcription factors(20), E26 transforming-specific sequence (ETS) transcription factors (21) and epigenetic regulatory mode(22). The results obtained in the studies of transcriptional genomics using microarrays associate several FOX transcription factors (FOXC1, C2, P1, P4 and O1A) with human heart failure, suggesting the pathophysiological significance of this gene family(23). In this review, we focus on analyzing the roles of FOX transcription factors identified in vascular pathology, diabetes and immunology. We apologize for not being able to include many valuable articles and reviews due to limited space.

3. OVERVIEW OF THE FOX TRANSCRIPTION FACTOR SUPERFAMILY

3.1. Structure

Since the discovery of Drosophila transcription factor fork head(24) and subsequent identification of the forkhead DNA-binding domain in the 1990s(25), more than 100 FOX genes and 19 human subgroups have been identified (FOXA to FOXS)(26). Subgroups are designated by a letter, and within each subgroup proteins are given a number(27). The FOX transcription factors are termed using the following convention: all capital letters for human (e.g. FOXA1); only the first letter capitalized for mouse (e.g. Foxa1); and the first letter and subgroup capitalized for all other chordates (e.g. FoxA1)(27). Members of this family have three α helices and two large loops or butterfly-like 'wings'(27). Therefore, the forkhead domain with approximately 100 amino acids is sometimes referred to as the forkhead/winged helix domain(27). The FoxO subgroup has received the most attention because of its recently discovered roles in reactive oxygen species (ROS) detoxification(28)(31), cell cycle progression(32), apoptosis(29, 30), cell size(17, 31-33), DNA repair(32), glucose metabolism(34, 35)(39) and vascular homeostasis(36). Given such extensive characterization, the FoxO subgroup will serve as a basis for introduction to the FOX transcription factor family.

3.2. Expression

FoxO protein expression has been reported in the ovary, prostate, skeletal muscle, brain, heart, lung, liver, pancreas, spleen, thymus and testis. However, in different cell types or organs, the expression levels of those FoxOs can differ considerably(27). FoxO1 is highly expressed in adipose tissue, whereas FoxO4 is highly expressed in muscle and FoxO3a in liver(42). FoxO6 expression appears to be restricted to brain(37). FOXO1, FOXO2, FOXO3a and FOXO4 are identified in fusion genes derived from chromosomal translocations in human soft tissue tumors and leukemias. FOXO1 is known as forkhead in rhabdomyosarcoma (FKHR), FOXO3a is termed as FKHR like protein 1 (FKHRL1), FOXO4 is known as AFK, an acute leukemia fusion gene located in chromosome X, and a fusion between FOXO2 and MLL occurs in some cases of acute myeloid leukemia(26).

3.3. Functional modes of FOX transcription factors

FoxO transcription factors must bind to DNA to either activate or repress target gene expression. They preferentially bind to DNA at the "FoxO-recognized element," which has the core consensus sequence 5'-T/C-G/A-A-A-A-C-A-A-3'. Fourteen protein-DNA contacts occur in the forkhead domain with the primary recognition site located at α-helix H3(26). Both the first and second loops of FoxO proteins make contact with DNA, but it is the second loop that can enhance the specificity and stability of the binding(26). The mechanisms underlying the binding of FoxO proteins to DNA have not been completely defined. FOX transcription factors may interact with a variety of cofactors such as CBP/p300, Smad (a class of transcription factors that modulate the activity of transforming growth factor (TGF)-β ligands), STAT (the

Signal Transducers and Activators of Transcription protein), PPAR (the peroxisome proliferator-activated receptors), Runx (a transcription factor that controls the timing of gene activation/inactivation), p53 (a tumor suppressor), other FOX transcription factors, and nuclear receptors for androgens, glucocorticoids, thyroid hormone, and retinoic acid. Such interactions may change the FOX transcription factors' DNA binding ability, thereby affecting their ability to promote or repress target gene expression(26, 38). FoxO transcription factors contribute to cardiac muscle remodeling and insulin signaling, and link insulin resistance with maladaptive heart hypertrophy. Calcineurin is an important phosphatase that activates a cascade of gene regulation through the nuclear factor of activated T cell 1 (NFAT1) transcription factors. The heterotrimeric protein phosphatase 2 (PP2, formerly PP2A) is a ubiquitous and conserved serine/threonine phosphatase with broad substrate specificity and diverse cellular functions. Sustained activation of FoxO1 or FoxO3 in cardiomyocytes selectively enhances the activity of protein kinase B (Akt) and reduces insulin signaling through inhibition of calcineurin and PP2(39, 40).

3.4. Combinatorial regulation of gene expression by FOX transcription factors and other transcription factors

In addition to working with other co-factors, FOX transcription factors often fulfill their function in regulating gene expression in combination with other transcription factors. Vascular development begins when mesodermal cells differentiate into endothelial cells, which then form primitive vessels. A 44 bp transcriptional enhancer in the genes' promoter regions is sufficient to direct gene expression specifically and exclusively to the developing vascular endothelium. This enhancer is regulated by a composite cis-acting element, the FOX:ETS motif, which is bound and synergistically activated by FOX and ETS transcription factors. Coexpression of FoxC2 and the ETS protein Etv2 induces ectopic expression of vascular genes in Xenopus embryos. Combinatorial knockdown of the orthologous genes in zebrafish embryos disrupts vascular development. Finally, FOX:ETS nucleotide sequence motifs are present in many known endothelial-specific enhancers, indicating that this motif is an efficient predictor of endothelial enhancers in the human genome(41). The interaction of FoxO with other transcription factors such as nuclear factor-κB (NF-κB) likely contribute to the complexity of the synergy between vascular endothelial growth factor (VEGF) signaling and FoxO in the upregulation of numerous genes including matrix metalloproteinase-10 (MMP-10), vascular endothelial cell adhesion molecule-1 (VCAM-1), endothelial-specific molecule-1 (ESM-1), bone morphogenetic protein-2 (BMP-2) and CBP-interacting transactivator-2 (CITED-2)(42). HOXA13 is a transcription factor that plays a role on placental formation(43). FoxF1 promoters bind to HOXA13 and can use these bound promoter regions to direct gene expression, providing a functional vascular endothelial labyrinth necessary for embryonic growth and survival(43).

3.5. Post-translational modification

In addition to being regulated at transcriptional and translational levels, the activities of the FOX transcription factor family are also regulated post-translationally via phosphoryaltion by Akt, a serine/threonine kinase(44). FOXO1 has phosphorylation sites at residues Thr24, Ser256, and Ser319(44). FOXO4 has phosphorylation sites at residues Thr28, Ser193, and Ser258(44). These phosphorylation sites are not equally modified. Among the three phosphorylation sites of FOXO3a (Thr32, Ser253, Ser315), Akt preferentially phosphorylates Ser253(30). Mutation of the Akt phosphoacceptor amino acids to alanine residues on FOXO can render it resistant to Akt phosphorylation and enhances the transcriptional activity of FOXO, suggesting that phosphorylation by Akt inhibits the transcriptional activity of FOXO(45). After activation, Akt translocates to the nucleus and phosphorylates the FOXO transcription factor, which results in the export of FOXO into the cytosol(44). Alternatively, the phosphorylation of FOXO proteins results in their inactivation through cytoplasmic retention(44). In the absence of Akt activity or following the mutation of FOXO phosphorylation sites, FOXO is exclusively localized to the nucleus(44). The translocation of FOXO following Akt phosphorylation is associated with the protein 14–3–3(44). The 14–3–3 family of proteins functions by binding to its protein ligands in a phosphorylation-dependent manner. Two binding motifs of 14–3–3 proteins have been identified, namely RSXpSXP and RXY/FXpSXP(46), which are present in nearly all known 14–3–3 proteins. Akt phosphorylation of FOXO3a results in the association of FOXO3a with 14–3–3 protein and retention of FOXO3a in the cytoplasm, rendering it ineffective to target genes in the nucleus and thus blocking its pro-apoptotic role(44). In addition, translocation of FOXO can also occur in response to a cellular insult, such as oxygen or glucose deprivation(44). FoxO factors can also be phosphorylated by other cellular kinases including NF-κB inhibitor (IκB) kinase, serum-glucocorticoid-regulated kinase, casein kinase 1, dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A, cyclin-dependent kinase 2 and mammalian Ste20-like kinase(38).

Some phosphorylation events can also regulate FoxO activity independent of cytosol-nuclear shuttling. For example, the phosphorylation mediated by Ras-related GTPase 9Ral-Jun kinase, cyclin-dependent kinase(47) and other mitogen-activated protein kinase pathways(38) modulate FoxO activity. In addition to phosphorylation, FOXO factors are also tightly regulated by other post-translational modifications including cAMP response element-binding protein (CBP)/p300-mediated acetylation(38), ubiquitin ligase Skp2-mediated ubiquitination(48) and deubiquitinase USP7-mediated deubiquitination(38).

In addition to being regulated by Akt, FoxO3a can also regulate the activation of Akt and extracellular signal-regulated kinases (ERK) as a feedback pathway. Foxo3a-deficient mice develop marked neutrophilia with age or during hematopoietic recovery after myelosuppressive stress induced by 5-fluorouracil (5-FU),

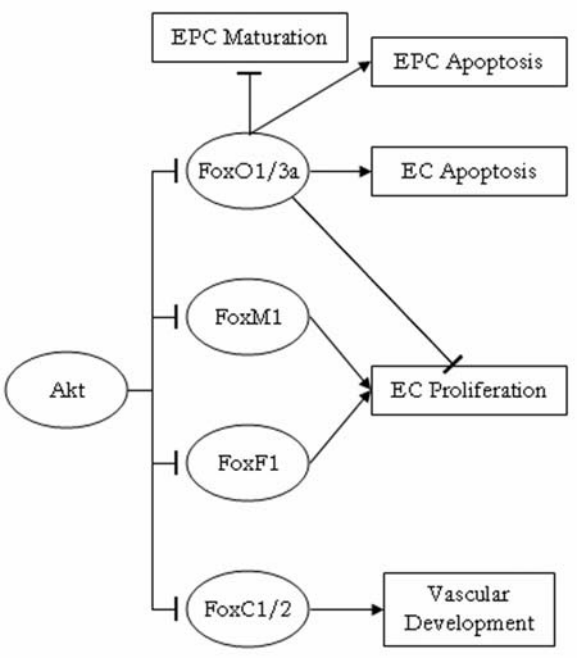

Figure 1. FOX transcription factors in endothelial cells. Various FOX transcription factors affect endothelial cell (EC) and endothelial progenitor cell (EPC) growth and apoptosis.

an antitumor chemotherapeutic drug, in a cell-autonomous manner. Akt and ERK activation are evident in hematopoietic stem cells (HSCs) of 5-FU-treated or aged Foxo3a$^{-/-}$-deficient mice. FoxO3a$^{-/-}$-deficient cells are hyperresponsive to cytokine stimulation, a phenotype effectively reversed by treatment with inhibitors of the Akt-mammalian target of rapamycin (mTOR) pathway or the MEK (mitogen-activated protein kinase kinase)-ERK pathway, indicating that FoxO3a plays a pivotal role in maintenance, integrity, and stress resistance of HSCs through negative feedback pathways for proliferation(49).

4. VASCULAR PATHOLOGY OF MICE DEFICIENT IN FOX TRANSCRIPTION FACTORS

Some members of the FOX transcription factor family play essential roles in development and differentiation of cardiovascular system(17). The gene knock-out mice of two subgroup C members, Foxc1$^{-/-}$ and Foxc2$^{-/-}$, show either embryonic or perinatal lethality, profound defects with aortic arch interruption, valve dysplasias, ventricular septal defects and absence of lymphatic valves. Compound Foxc1$^{-/-}$/Foxc2$^{-/-}$ knock-out mice show embryonic lethality around 9 days postcoitum, disorganized mesodermal patterning, abnormal remodeling of vascular plexi, deficient arterial specification, absence of outflow tract and incomplete cardiac morphogenesis(17). In addition, Foxf1$^{-/-}$ knock-out mice show embryonic lethality around 8.5 days postcoitum with absence of vasculization in the yolk sac and allantois(17). In Foxh1$^{-/-}$ knock-out mice, embryos lack midline structures, and the absence of anterior heart fields results in malformations of the outflow tract and the right ventricle(17). In Foxm1$^{-/-}$ deficient mice, aberrant cardiomyocyte polydiploidy attributes to irregular re-replication. Lung microvasculature also shows defective formation in these mice(17). In Foxo1$^{-/-}$ deficient mice, impaired angiogenesis is evident around E9.5 (days in the embryogenesis) with defects found in the dorsal aorta, branches of the carotid arteries and intersomitic and yolk sac vessels(17). Foxo3$^{-/-}$ deficient mice show enhanced formation of vessels following hind limb ischemia and enhanced hypertrophic growth of the heart at baseline(17). Foxo4$^{-/-}$ deficient mice exhibit reduced intimal hyperplasia following carotid artery ligation(17). The deletion of all six alleles in adult compound deficient Foxo1$^{-/-}$/Foxo3$^{-/-}$/Foxo4$^{-/-}$ mice results in the development of hemangiomas in tissues such as the liver, skeletal muscle, bone marrow, abdominal wall and uterus. However, tumors do not form in the lung or kidney(17). These results demonstrate that the mammalian FoxOs are indeed *bona fide* tumor suppressors. Transcriptome and promoter analyses of differentially affected endothelium identify direct FoxO targets and reveal that FoxO regulation of these targets *in vivo* is highly context-specific, even in the same cell type. Functional studies have validated two proteins Sprouty2 and PBX1, among others, as FoxO-regulated mediators of endothelial cell morphogenesis and vascular homeostasis(41). Foxp1$^{-/-}$ deficient mice show embryonic lethality at E14.5 attributable to aberrant cardiac development. Defects include reduced thickness of myocardium, valve dysplasias and insufficient outflow tract septation(17). We reason that the similarity of vascular phenotypes in mice deficient of some Fox transcription factors suggests that these factors have functional redundance. Although some FOX factors in the same subgroup share sequence homology in the forkhead domain, the sequence differences in the regions outside and inside of the forkhead/wingled helix domain may be responsible for diversified vascular functions.

5. ROLES OF FOX TRANSCRIPTION FACTORS IN ENDOTHELIAL CELL PATHOLOGY

The work of Wang's laboratory suggests that the roles of risk factor(s) in atherogenesis are related to endothelial damage caused by the risk factor(s)(5)(6). It is seen that endothelial damage is a main mechanism underlying the pathogenesis of atherosclerosis, restenosis and posttransplant graft atherosclerosis in addition to infiltration of inflammatory cells and proliferation of smooth muscle cells. Blocked apoptosis of endothelial cells results in significant reduction of intimal hyperplasia *in vivo*(50). FOX transcription factors fit into this paradigm according to Figure 1, which will serve as a useful reference for the ensuing discussion.

5.1. Endothelial apoptosis

In vascular endothelial cells, Foxo1 and Foxo3a are pro-apoptotic proteins regulated by Akt(51-53). Upon activation by VEGF or insulin/insulin-like growth factor-1 (IGF1), Akt mediates pro-survival and anti-apoptotic signaling in part via the phosphorylation and inactivation of FoxO transcription factors(34). Akt-regulated FOXO3a controls endothelial cell viability through modulation of the

expression of caspase-8 inhibitor FLIP (FLICE-inhibitory protein)(54). FLIP is a homologue of caspase-8 that lacks catalytic activity and has been shown to be important in protecting endothelial cells from apoptosis(54). Akt promotes FLIP expression in endothelial and tumor cells(60). Transduction of a nonphosphorylatable, constitutively active mutant of FOXO3a (TM-FOXO3a) down-regulates FLIP, increases caspase-8 activity and promotes apoptosis in endothelial cells(54). Conversely, transduction of a dominant-negative mutant of FOXO3a up-regulates FLIP levels and protects endothelial cells from apoptosis induced by serum deprivation conditions(54). Restoration of intracellular FLIP blocks caspase-8 activation and inhibits apoptosis in TM-FOXO3a-transduced cells(54). This establishes a convincing mechanism for FOX-mediated endothelial cell apoptosis.

5.2. EPC apoptosis and maturation

FOXO may contribute to endothelial progenitor cell (EPC) apoptosis. EPCs are present in the systemic circulation and home in on sites of ischemic injury(55, 56). Circulating EPC levels are an indicator of cardiovascular health(57, 58). Phosphorylation and therefore inactivation of FOXO4 by statins prevents EPC apoptosis(65). In addition, statins reduce the expression of the pro-apoptotic FOX-regulated protein Bim in a phosphatidylinositol 3-kinase (PI3K)-dependent manner(59). Similarly, scleroderma serum-induced EPC apoptosis is mainly mediated by the Akt-FOXO3a-Bim pathway, which may account, at least in part, for the decreased circulating EPC levels in scleroderma patients(60). Moreover, Akt expression is attenuated in the early stages of differentiation and is gradually upregulated during EPC maturation. FOXO3a, an Akt downstream target, is downregulated through phosphorylation in the late stages of EPC differentiation. Adenovirus-mediated overexpression of activated FOXO3a in peripheral blood mononuclear cells markedly increases the number of cell foci but reduces the number of Di-acetyl LDL-expressing EPCs that appear at later time points. These data suggest that Akt/FOXO3a signaling is an important regulator of EPC maturation(61).

5.3. Endothelial cell proliferation

FOX transcription factors modulate the migration and proliferation of aortic endothelial cells, which are critical processes involved in atherosclerosis and postangioplasty restenosis(53). Inhibition of FOXO factors promotes endothelial proliferation by down-regulating p27Kip1, a member of the universal cyclin-dependent kinase inhibitor family(62). Transfection of endothelial cells with constitutively active TM-FOXO3a up-regulates p27Kip1, whereas transfection with a constitutively active Akt decreases p27Kip1 expression(62). Reducing FOXO expression using RNA interference attenuates p27Kip1 expression and stimulates endothelial cell proliferation(62). In contrast, targeted inactivation of FoxF1 leads to loss of vasculature(63). FoxF1 is crucial for assembly of endothelial cells into simple tubes from clusters of mesodermal angioblasts(63). Similarly, endothelial cell-restricted disruption of FoxM1 impairs endothelial repair following bacterial toxin lipopolysaccharide (LPS)-induced vascular injury(64). Endothelial cells isolated from endothelial cell-restricted Foxm1-deficient mouse lungs fail to proliferate, and small interfering RNA (siRNA)-mediated suppression of FOXM1 in human endothelial cells results in defective cell cycle progression(64). These data suggest differential roles of FOX proteins in endothelial cell proliferation.

Another mode by which FOX transcription factors may regulate endothelial cell proliferation is downstream of homocysteine (Hcy)-induced effects. Wang's laboratory and others confirmed that HHcy acts as an independent risk factor in accelerating atherosclerosis(4-8). High levels of Hcy induce a sustained injury of arterial endothelial cells, which accelerates the development of thrombosis and atherosclerosis. In addition, Hcy specifically inhibits the growth of endothelial cells. Hcy induces dephosphorylation of Akt and FOXO3a and upregulates p27Kip1 in a time- and dose-dependent manner. PI3K activator peroxovanadate (PV) and PP2 inhibitor okadaic acid can reverse the Hcy inhibition of endothelial growth. Pretreatment with PV and okadaic acid prevents Hcy-induced cell cycle G1 phase arrest. Transfection with specific antisense oligonucleotides to Akt further supports these observations. These results suggest a new pathogenic mechanism underlying HHcy as an independent risk factor for cardiovascular diseases(65).

5.4. Roles of FOX transcription factors in VEGF signaling

FoxC1 and FoxC2 are essential for arterial cell specification during development. In the developing embryo, arterial and venous identity is established by genetic mechanisms before circulation begins. VEGF signaling and its downstream Notch pathway play critical roles in arterial cell fate determination. FoxC1 and FoxC2 directly induce the transcription of Delta-like 4 (Dll4), a ligand for Notch receptors. FoxC2 physically and functionally interacts with a Notch transcriptional activation complex containing Su(H) and Notch intracellular domain to induce Hey2 promoter activity. FoxC transcriptional factors interact with VEGF and Notch signaling to regulate arterial gene expression in multiple steps of the VEGF-Dll4-Notch-Hey2 signaling pathway(66). In addition, VEGF is a direct transcriptional target of FoxM1b. In glioma cells, FoxM1b overexpression increases VEGF expression, whereas blockade of FoxM1b expression suppresses VEGF expression(67).

Incubation of human coronary artery endothelial cells with hepatocyte growth factor (HGF) induces prolonged PI3K/Akt-dependent phosphorylation and nuclear exclusion of FOXO1. HGF-mediated inhibition of FOXO1 activity results in secondary attenuation of VEGF-induced expression of FOXO1-dependent genes including VCAM-1, manganese superoxide dismutase (MnSOD), ESM-1, CBP/p300 interacting transactivator with ED-rich tail-2, BMP-2, MMP-10, and MGC5618(68). Foxo1-deficient mice have also been ascribed to have an insufficient endothelial response to VEGF. FoxO1-deficient yolk sacs show reduced expression of essential endothelial gap junction connexins 37 and 40 and ephrin-

B2 (a ligand for the Eph receptor responsible for vascular patterning and identity)(17). Postnatal deletion of the FoxO1 gene by the transient activation of Cre recombinase through the Mx1 promoter results in the appearance of hemangiomas, but deficiencies of FoxO3 and/or FoxO4 do not recreate the tumor phenotype observed in Foxo1 deficient mice. These results suggest that in endothelial cells, FoxO1 is the dominant factor in suppressing tumor formation(17).

5.5. Endothelial responses to stress

FOX transcription factors have specific responses under conditions of stress. Prolonged shear stress (18 hours) leads to a significant (50%) decrease in hydroxyl-methylglutaryl coenzyme A reductase (HCR) mRNA expression via the phosphorylation and degradation of FoxO1a(69). HCR is the rate-limiting enzyme for cholesterol synthesis(69). Correspondingly, the downregulation of FoxO with siRNA decreases HCR expression(69). In addition, angiotensin II (Ang II) is a powerful accelerator of atherosclerosis and modulates the expression of endothelial nitric oxide synthase (eNOS)(70). Exposure of human umbilical vein endothelial cells to Ang II elicits a rapid phosphorylation of Akt and FoxO1(76). Constitutively active Akt inhibits the promoter activity of a scavenger receptor of the BI class (hSR-BI/CLA-1), whereas a dominant-negative mutant of Akt or mutagenesis of a FoxO1 response element in hSR-BI/CLA-1 abolishes the ability of Ang II to suppress promoter activity(70). Thus FoxO1 mediates two distinctive outcomes in response to stress.

5.6. Neovascularization

eNOS, which is essential for postnatal neovascularization, is regulated by FoxO1 and FoxO3a(77). EPCs promote neovascularization in sites of ischemic injury(55, 56). Constitutively active FoxO1 and FoxO3a repress eNOS expression by binding to the eNOS promoter(71). *In vivo*, FoxO3a deficiency increases eNOS expression and enhances postnatal vessel formation and maturation(77). BMPs are involved in embryonic and adult blood vessel formation in health and disease. BMPER (BMP endothelial cell precursor-derived regulator) is a differentially expressed protein in embryonic endothelial precursor cells. BMPER is a downstream target of FoxO3a and consistently exerts activating effects on endothelial cell sprouting and migration *in vitro* and *in vivo*(72). Intercellular junctions mediate adhesion and communication between adjoining cells. Although formed by different molecules, tight junctions (TJs) and adherens junctions (AJs) are functionally and structurally linked. Vascular endothelial (VE)-cadherin at AJs upregulates TJ adhesive protein claudin-5. This regulation requires alleviation from inhibition by FoxO1 and the T cell factor (Tcf)-4-β-catenin transcriptional repressor complex. VE-cadherin acts by inducing the phosphorylation of FoxO1 through Akt activation and by limiting the translocation of β-catenin to the nucleus(73). Collectively, the studies of FOX transcription factors in endothelial cells show a myriad of distinct roles for development, proliferation, and cell death as summarized in Figure 1.

6. ROLES OF FOX TRANSCRIPTION FACTORS IN VASCULAR SMOOTH MUSCLE CELLS (VSMCS)

6.1. VSMC apoptosis

FoxO transcription factors have roles in regulating VSMC apoptosis. VSMCs are essential for the structural integrity and contractile responses of the arterial vessel wall(74). During the early phase of atherogenesis, the proliferation of VSMCs in response to inflammatory stimuli dominantes over VSMC apoptosis(16). As with endothelial cells, apoptosis of VSMCs is an important regulator of the stability of atherosclerotic plaques(74). Blockage of phosphorylation of FoxO3 correlates with increased VSMC apoptosis(75). FoxO transcription factors can modulate VSMC cell surface expression of CD95 (Fas) ligand (FasL), which is an important determinant for cell death(76). Ectopic expression of FoxO3 in VSMCs induces FasL expression and DNA fragmentation, which is partially dependent on the activity of caspase-8(76). Brunet *et al.* identified three putative overlapping FoxO3 response elements in the FasL promoter, two of which are found to bind FoxO3(30).

6.2. VSMC proliferation

FoxO transcription factors also regulate VSMC proliferation. VSMC proliferation and survival are implicated in vascular diseases such as restenosis following angioplasty or stenting. Inactivation of FOX transcription factors can lead to transcriptional down-regulation of p27Kip(77-79). Down-regulation of p27Kip1 is associated with increased cell cycle entry(78). FOX transcription factor inactivation and p27Kip1 down-regulation are prevented by one of the following approaches: (1) inhibition of PI3K with wortmannin or LY294002; (2) overexpression of a constitutively inactive form of Akt; or (3) overexpression of constitutively active forms of FOX transcription factors(77-79). The anti-proliferative effect of TM-FOXO3 can also be partially reversed by siRNA against p27Kip1(77). In the carotid artery balloon injury model, TM-FOXO3 delivered by adenovirus to arteries decreases the proliferation of VSMCs and reduces the intima/media ratio with an accompanying increase of p27Kip(77, 79). Recent evidence also suggests that the upregulation of p27Kip may not be the only mechanism by which FoxO inhibits VSMC proliferation(17). The cysteine-rich protein 61 (CYR61, CCN1), an immediate early gene and a potent angiogenic factor rapidly expressed and secreted from VSMCs after angioplasty or Ang II stimulation. CYR61 has been shown to be negatively regulated by FoxO in VSMCs(80). CYR61 is an extracellular matrix-associated protein that can interact with integrins to promote VSMC migration and adhesion. CYR61 has been implicated in processes such as atherosclerosis and vascular resenosis(81-83). A functional association between FoxO and CYR61 is first noted after identification of a forkhead binding element in the promoter of the CYR61 gene(17). Adenoviral delivery of TM-FOXO3 suppresses CYR61 expression, inhibits proliferation, and reduces cell viability(74, 80). This repression seems to work via a direct effect because FOXO3a is detected at the CYR61 promoter by chromatin immunoprecipitation(74). Moreover, a reporter assay

shows that deletion of the FOXO binding site in the CYR61 promoter abrogates the repression of CYR61 expression by TM-FOXO3a(74). Conversely, concomitant delivery of adenoviruses expressing CYR61 and TM-FOXO3a reverses the intima-sparing effect of TM-FOXO3, inhibits FOXO3a-induced cellular detachment and reduces viability *in vitro* without affecting the proportion of cells in the sub-G1 (presumably apoptotic) population identified by propidium iodide apoptotic cell staining(74, 80). Epidermal growth factor ligand, such as betacellulin (BTC), induces the phosphorylation of FoxO1 and FoxO4, in a dose- and time-dependent manner(84). FoxO4 localization in the nuclei of cultured aortic smooth muscle cells is associated with reduced expression of myogenic markers(17). Myogenic differentiation-specific genes that are likely to be repressed by nuclear FoxO4 include SMα-actin, SM-calponin, and SM-22α(17). In agreement with this model, the nuclear translocation of FoxO4 is found to occur in proliferating VSMCs after *in vivo* vascular injury(85). Cyclin D1 is a key regulator of cell proliferation that promotes the progression of G1 phase. Recently, a correlation between FoxO phosphorylation and cyclin D1 expression has been found in the regulation of VSMC proliferation(84). BTC, which increases the expression of cyclin D1 in VSMC, induces the phosphorylation of FoxO via the PI3K/Akt signaling pathway. This indicates that the up-regulation of cyclin D1 induced by BTC in VSMC may be caused by the releasing the repression of FoxO factors.

6.3. FoxO in VSMC differentiation

FoxO4 has been shown to modulate the transition of VSMCs from a contractile to a more proliferative phenotype(86). Myogenic differentiation-specific genes are repressed by nuclear FoxO4. These genes are under positive regulation of myocardin(87), a transcriptional coactivator that is essential for the induction of differentiation in VSMCs(88, 89). The interaction of FoxO4 with myocardin in cultured aortic smooth muscle cells was shown to repress the differentiation of VSMCs initiated by myocardin(85). The nuclear translocation of FoxO4 was found to occur in proliferating VSMCs after *in vivo* vascular injury(85). This suggests that nuclear prevalence of FoxO4 is necessary to suppress the differentiation program and promote a dedifferentiated and more proliferative phenotype.

6.4. FoxO in aging VSMCs

In VSMCs of old rats, phosphorylated FoxO3a is increased(90). MnSOD is one of the major cellular antioxidant defense systems and a recent study showed that both MnSOD protein expression and activity are reduced in VSMCs from old animals as compared to that from young animals. FoxO3 interacts with the promoter of the rat MnSOD gene and inhibition of FoxO3a transcription leads to reduction of MnSOD expression.

One of Akt's VSMC protective effects has been identified as its inhibition of FoxO3a or glycogen synthase kinase-3 (GSK3) by phosphorylation. Activation of IGF1R, which is increased in the VSMCs of old animals, leads to the activation of Akt and FoxO3a. Genes for p27Kip, catalase, and MnSOD, which play important roles in the control of cell cycle arrest and stress resistance, are found to be FOXO3a targets. IGF1R signaling modulates these genes through activation of the Akt/FOXO3a pathway(91). The deregulation of the Akt-FoxO3a-GSK3 pathway, due to a reduction of IGF1R signaling, promotes apoptosis in atherosclerosis(92).

6.5. Roles of other FOX transcription factors in VSMCs

In addition to FoxO, other forkhead transcription factors are also involved in VSMC survival. Lungs of $Foxm1^{-/-}$ mice exhibit severe hypertrophy of arteriolar smooth muscle cells and defects in the formation of peripheral pulmonary capillaries as evidenced by significant reduction in the staining of capillary-marker platelet endothelial cell adhesion molecule 1 in the distal lung(93). Premature expression of the FoxM1b transgene protein accelerates proliferation of different lung cell types, including endothelial cells of pulmonary capillaries and arteries(94). Thus FOX transcription factors have important roles in multiple types of cardiopulmonary smooth muscle.

7. ROLES OF FOX TRANSCRIPTION FACTORS IN THE PATHOGENESIS OF DIABETES

Diabetes is a disease defined by abnormalities of fasting or postprandial glucose and is frequently associated with disorders of the eyes, kidney, nerves and cardiovascular system. Diabetes generally results in early death from cardiovascular diseases. Thus, some of the roles of FOX transcription factors in diabetes are associated with their roles in the pathogenesis of vascular diseases(95).

7.1. FoxO and diabetes

FoxO transcription factors are involved in several pathways responsible for the onset of diabetes mellitus (DM) and diabetic complications(26). In calorie restriction or starvation, FoxOs have transcriptional activity in the nucleus, where their effects increase hepatic glucose production, decrease insulin secretion, increase food intake and cause degradation of skeletal muscle in order to supply substrates for glucose production. Even with insulin resistance due to excessive caloric intake, FoxOs are active and cause type 2 diabetes and hyperlipidemia. The understanding of the molecular mechanisms by which FoxOs affect glucose or lipid metabolism will shed light on a novel therapy for type 2 diabetes and metabolic syndrome(96). FoxO1, which is phosphorylated and inhibited by Akt, plays an important role in insulin signaling(97). The physical association between the carboxyl terminus (amino acids 1280-1499) of tuberous sclerosis protein 2 (TSC2, also known as tuberin) and FoxO1 degrades the TSC1-TSC2 complex, which results in the activation of mTOR and subsequent phosphorylation of the main mTOR substrate, ribosomal p70-S6 kinase (p70-S6K)(98). p70-S6K may be implicated in a negative feedback loop to suppress insulin signaling(97). p70-S6K has an inhibitory effect on Akt activation downstream of insulin receptor(97). p70-S6K enhances insulin receptor (IR) substrate (IRS-1) serine phosphorylation and

degradation, leading to decreased Akt phosphorylation and insulin resistance(99). Prolonged overexpression of wild type FoxO1 enhances phosphorylation of the Ser307 residue of IRS-1 and decreases phosphorylation of Akt and FoxO1 itself even in the presence of serum(97). These data suggest that FoxO1 regulates the insulin signaling pathway(97). Additional investigations have associated diabetic nephropathy to post-translational changes in FoxO3a. Phosphorylation of FoxO3a is increased in rat and mouse renal cortical tissues two weeks after the induction of diabetes by the β-cell chemotherapy drug streptozotocin(100). Furthermore, enteric neurons can be protected from hyperglycemia by glial cell line-derived neurotrophic factor, which can affect Akt signaling and prevent FoxO3a activation(101). Interestingly, Akt inhibits expression of pyruvate dehydrogenase kinase-4, a protein that conserves gluconeogenic substrates during DM and requires the inhibition of FoxO3a activity(102). In addition, the human immunodeficiency virus-1 accessory protein Vpr has been reported to contribute to insulin resistance by interfering with FOXO3a signaling with protein 14-3-3 in human hepatoma cells(103). Furthermore, loss of Foxo1 in the liver of mice leads to impaired glycogenolysis and gluconeogenesis, which suggests an important role for FoxO1 in regulating glucose production(104). Thus FoxO transcription factors have roles in multiple aspects of glucose metabolism.

7.2. FoxO, EPC and diabetes

A recent report has also established a link between glucotoxicity and FoxO activity in EPCs(105). Recent advances in the study of EPCs suggest that EPCs isolated from diabetic patients are largely dysfunctional(58, 106). Dysfunction of mature endothelial cells is thought to play a major role in both the micro- and macrovascular complications of diabetes(105). Increased levels of glucose induce a distinct pattern of post-translational modifications of FoxO1, characterized by reduced phosphorylation at Akt consensus sites and enhanced acetylation of lysine residues(17). Under these conditions, FoxO1 nuclear localization is accompanied by up-regulation of the pro-apoptotic factors Bim and FasL(17). Indeed, EPCs treated with high levels of glucose show increased rates of apoptosis, and this effect is reversed by benfotiamine, a thiamine derivative, via Akt/FoxO1(105, 107). Therefore, aberrant activation of FoxO factors under conditions of glucose toxicity may be associated with the impaired ability of EPCs to differentiate and promote *de novo* tube formation(105). In EPCs challenged with high doses of glucose, FoxO factors are intimately associated with induction of apoptosis, which can be at least in part inhibited by active PI3K/Akt signaling(17). Similarly, mammalian Sir2 (SIRT1) deacetylase mediates caloric restriction and influences lifespan by regulating a number of biological molecules, such as FoxO1. SIRT1 controls the angiogenic activity of endothelial cells via deacetylation of FoxO1. Endothelial dysfunction and reduced new blood vessel growth in diabetes involve decreased bioactivity of EPCs via repression of FoxO1 transcriptional activity. Treatment of EPCs with high glucose for 3 days results in a consistent reduction of DiLDL/lectin staining and, interestingly, this is associated with reduced SIRT1 expression and activity, and with increased acetyl-FoxO1 levels. These results suggest that FoxO1 mediates its effects via SIRT1, a critical modulator of EPC dysfunction during alteration of glucose metabolism(108).

7.3. FoxO and energy metabolism

FoxO proteins are also closely linked to the prevention of diabetic complications through the preservation of cellular energy reserves and mitochondrial integrity(26). However, the role of FoxO proteins in maintenance of cellular energy reserves is not completely clear because some studies show that overexpression of Foxo1 in mouse skeletal muscle can lead to reduced skeletal muscle mass and poor glycemic control(109). FOX transcription factors also play a significant role in regulating whole body energy metabolism. Glucose homeostasis is achieved by adjusting endogenous glucose production as well as glucose uptake by peripheral tissues in response to insulin. In the fasting state, the liver is primarily responsible for maintaining glucose levels, with FoxO1 playing a key role in promoting the expression of gluconeogenic enzymes. Following feeding, pancreatic β-cells secrete insulin, which promotes the uptake of glucose by peripheral tissues including skeletal muscle and adipose tissue. This can in part suppress gluconeogenic enzyme expression in the liver. In addition to directly regulating metabolism, FoxO1 also plays a role in the formation of both adipose tissue and skeletal muscle, two major components critical for maintaining energy homeostasis. The importance of FoxO1 in energy homeostasis is particularly striking under conditions of metabolic dysfunction or insulin resistance. In obese or diabetic states, FoxO1-dependent gene expression promotes some of the deleterious characteristics associated with these conditions, including hyperglycemia and glucose intolerance. In addition, the increase in pancreatic β-cell mass that normally occurs in response to a rise in insulin demand is blunted by nuclear FoxO1 expression. However, under these same pathophysiological conditions, FoxO1 expression may help drive the expression of genes involved in combating oxidative stress, thereby preserving cellular function. FoxO1 may also be involved in promoting the switch from carbohydrates to fatty acids as the major energy source during starvation(110). Though it remains to be shown, the myriad of roles for FoxO proteins in initiating, propagating, and preventing metabolic disease may be associated with potential phase-specific expression patterns of FOX factors.

8. IMMUNE SYSTEM PHENOTYPES OF MICE DEFICIENT IN FOX TRANSCRIPTION FACTORS

Recent studies performed on hypercholesterolemic mice deficient in different components of the immune system uniformly suggest that the net effect of immune activation is pro-atherogenic and that atherosclerosis, at least to some extent, should be regarded as an autoimmune disease(3, 16, 111, 112). Therefore, some of the roles of FOX transcription factors in the pathogenesis of cardiovascular diseases are associated with their roles in the regulation of immune responses and

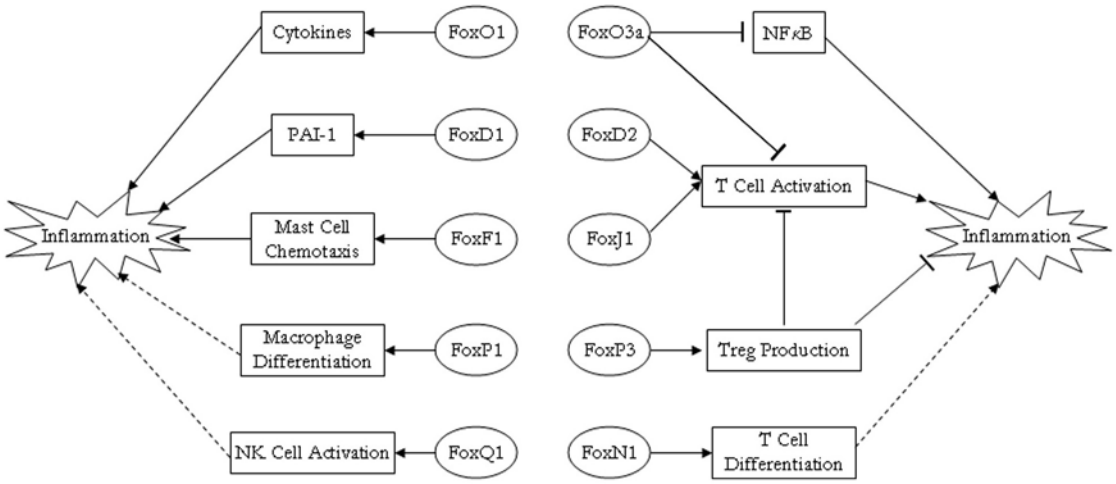

Figure 2. FOX transcription factors in immune responses and inflammation. Various FOX transcription factors mediate immune responses that regulate inflammation. Dashed lines represent pathways that do not necessarily produce inflammation. PAI-1: Plasminogen Activator Inhibitor-1; NF-κB: Nuclear Factor-κB.

inflammation. In addition, it becomes clear that these FOX transcription factors also play crucial roles in various aspects of immune regulation. Several members of the FOX transcription factor family, for example FOXF1, FOXP3, FOXN1, FOXO1 and FOXO3, have been shown to execute diverse functions in regulating inflammation and adaptive immune response(11, 38, 113, 114) . Figure 2 will serve as a reference for our following discussion.

8.1. FOX transcription factors and inflammation

FOX transcription factors play important roles in vascular inflammation. FoxF1 (previously known as HFH-8 or Freac-1) is expressed in endothelial and smooth muscle cells in the embryonic and adult lung. Haploinsufficient $Foxf1^{+/-}$ mice develop severe airway obstruction and bronchial edema associated with increased numbers of pulmonary mast cells and increased mast cell degranulation after injury. Pulmonary inflammation in $Foxf1^{+/-}$ mice is associated with diminished expression of Foxf1, increased mast cell tryptase, and increased expression of CXCL12, the latter being essential for mast cell migration and chemotaxis. Foxf1 haploinsufficiency causes pulmonary mastocytosis and enhanced pulmonary inflammation after chemically induced or allergen-mediated lung injury, indicating Foxf1 plays an important role in inhibiting the pathogenesis of pulmonary inflammatory responses via suppressing mast cell migration and chemotaxis(115). It has been reported that mast cells promote atherogenesis and induce destabilization in apolipoprotein E-/- deficient mice(116). Thus, Foxf1 may play an important role in suppressing roles of mast cells in atherogenesis.

FoxD1 has a role in the induction of plasminogen activator inhibitor-1 (PAI-1), a serpin class protease inhibitor that plays a central role in the regulation of vascular function and tissue remodeling by modulating thrombosis, inflammation, and the extracellular matrix. A central mediator in controlling PAI-1 expression is immunosuppressive cytokine TGF-β, which induces PAI-1 expression and promotes fibrosis. Overexpression of Smad6s (an endothelial splice variant) from the Smad family of signal transduction molecules in endothelial cells increases PAI-1 promoter activity and secretion, whereas antisense Smad6s suppresses the induction of PAI-1 by TGF-β. The levels of Smad6s can alter the levels of TGF-β and the subsequent induction of PAI-1 via a FoxD1 transcription site. Further data suggests that this process, which is up-regulated in diseased vessels, can be modulated by the inhibition of protein kinase C (PKC)-β(117).

FoxO proteins may have a role in propagating the obesity-associated low-grade inflammation in adipose tissue that results from increased production of pro-inflammatory cytokines. Subsequently, increased proinflammatory cytokines can contribute to the development of insulin resistance. Tumor necrosis factor (TNF)-α treatment attenuates Akt-dependent phosphorylation of FoxO1 and enhances transcriptional activity of FoxO1. FoxO1 increases the expression of CCAAT/enhancer binding protein (C/EBPβ, a positive regulator of monocyte chemotactic protein (MCP)-1 and interleukin (IL)-6 genes) through directly binding to its promoter. These findings suggest that activation of FoxO1 triggered by TNF-α up-regulates the expression of C/EBPβ in 3T3-L1 adipocytes, thereby leading to an increased production of pro-inflammatory cytokines, MCP-1 and IL-6(118). However, unlike TNF-α, bacterial endotoxin LPS utilizes the PI3K pathway to inhibit FoxO3a. Inhibition of PI3K attenuates LPS-induced production of proinflammatory cytokine IL-8. LPS-induced IL-8 is increased in HT-29 cells with silenced FOXO3a. Moreover, in HT-29 cells with silenced FOXO3a, the expression level of IκBα, an NF-κB inhibitor, is decreased. Thus, LPS and bacterial infection inactivate FoxO3a in intestinal epithelia via the PI3K pathway and further suppresses IκBα, leading to the activation of NF-κB and subsequent upregulation of IL-8(119). Currently, it

remains unclear whether the roles of FOX factors in regulating proinflammatory cytokines either positively or negatively are cell-specific, cytokine specific, or inflammation phase specific.

8.2. FoxP3, regulatory T cells and immune suppression.

FoxP3 is by far the most intensely studied forkhead family member in immune regulation due largely to its roles in differentiation, homeostasis and suppression of CD4+CD25^{high} regulatory T cells (Tregs)(11). Tregs, characterized by high expression of CD25 (an IL-2 receptor α-chain), comprise 5-10% of the total population of CD4+ T cells in mice. Tregs downregulate the reactivity of CD4+CD25- T helper cells (Th cells) and play crucial roles in the suppression of inflammation, anti-tumor immune responses, autoimmune diseases and transplant rejection(11, 120-122). FoxP3 is highly expressed in Tregs in both humans and in mice(123, 124). The Scurfy mice have been identified to have defective Foxp3 genes. The function of Foxp3 is essential for normal immune homeostasis(125). The phenotypes in the Scurfy mice include lethality of hemizygous males 16-25 days after birth and overproliferation of activated CD4+ T cells with multi-organ infiltration(126). Interestingly, adoptive transfer of wild-type lymphocytes can control the T cell activation in the Scurfy mice and prevents autoimmune disease development(126), which indicates that the Scurfy mice lack a certain lymphoid compartment that can repress the activity of activated T cells. A variety of studies on patients' families also indicate that FOXP3 mutation leads to the immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) in humans(127). Patients with IPEX have massive T-cell infiltration into the skin and gastrointestinal tract as well as high levels of autoantibodies in serum. Foxp3 transgenic mice have increased numbers of Tregs, which strongly suppress the proliferation of antigen-stimulated CD4+ T cells. Transgenic CD4+ and CD8+ cells are hyporesponsive to activation. All of the disease manifestations and animal models support the crucial role of FoxP3 in Tregs' development. Most recently, Yang's laboratory and others showed that the survival/apoptosis pathway of Foxp3-controlled CD4+CD25^{high} Tregs modulate vascular inflammation(12, 13), diabetes(14) and atherosclerosis(128). These studies clearly suggest that Foxp3, through its function in differentiation and promotion of Tregs' homeostasis and suppressive function, suppresses inflammation and inhibits certain inflammatory cardiovascular diseases like atherosclerosis and hyperlipidemia. Tregs represent a safe and efficient source for therapy, and they could become an important weapon in the fight against immune mediated pathology(129).

8.3. FoxN1 and thymocyte development.

FoxN1 has an important role in epithelial cell development. FoxN1 mediates thymic epithelial cell differentiation both in mice and human. Thymic epithelial cells create a proper microenvironment in the thymus stoma for the development and selection of thymocytes (precursors of T cells)(130). The role of FoxN1 in thymic epithelial development makes it a focus for immunology research(131). Mouse nude mutations, eliminating the DNA binding domain of FoxN1, lead to defective differentiation of epithelial progenitor cells in the thymus(132). Consequently, these defective epithelial cells fail to attract lymphoid progenitors to the thymic anlage, and finally results in defective thymocyte/T cell development(133). Human nude/severe combined immunodeficiency (SCID) syndrome, consisting of T cell deficiency, congenital alopecia and nail dystrophy, is caused by a nonsense mutation in FOXN1(134). Even bone marrow transplantation cannot restore normal levels of CD4+ T cells in this syndrome, suggesting that defect resulted from FOXN1 mutation is thymus-derived but not bone marrow-derived.

8.4. FOX transcription factors and T cell activation.

Although the FoxO transcription factors have been widely studied for their metabolic and homeostatic roles, the immunological role of FoxO3a in suppressing spontaneous T cell activation and autoimmunity makes it a widely studied target in immunology. Interferon-γ driven tryptophan catabolism by cytotoxic T lymphocyte antigen 4 (CTLA-4) might activate FoxO3a to protect dendritic cells from injury in nonobese diabetic mice(135). At the transcriptional level, FoxO3a is the dominant isoform expressed in lymphocytes. Foxo3a$^{-/-}$ mice develop lymphoproliferative disease with multi-organ infiltrates, resulting in multisystem inflammation,enlarged spleen and lymph nodes due to the increased lymphocyte proliferation(136). Foxo3a deficient mice have spontaneous, autoreactive helper T cell activation. FoxO3a$^{-/-}$ deficient T cells possess increased spontaneous NF-κB activity, and are relatively deficient in the NF-κB inhibitors, IκBβ and IκBε subunits(113). FoxO3a can also regulate cell proliferation and apoptosis, both of which contribute to lymphocyte homeostasis. For example, FOXO3a has been shown to regulate cell division through a cyclin G2 dependent mechanism(137). Cyclin G2 has been shown to maintain the quiescent state of differentiated cells and negatively regulates lymphocyte proliferation. Activated FOXO3a has been shown to control the expression of some proapoptotic genes, for example, FasL. No human immunological diseases caused by FOXO3a defects have been clearly identified. However, there is FoxO3a dysregulation in cancers in which anti-cancer immunosurveillance is weakened. For example, mixed lineage leukemia transcription factor fusion proteins with FoxO3a have been identified in acute lymphoblastic leukemia(138). Many other forkhead transcription factors also play important role in the regulation of a variety of immunologic functions. FoxJ1 suppresses spontaneous T cell activation and autoimmunity(139). In animals with FoxJ1 (hepatocyte nuclear factor/forkhead homolog 4, HNF-4, FKHL-13) deficient lymphoid systems, Th spontaneously activate, resulting in multi-system inflammation, particularly of the lung, liver, kidney, and salivary glands. Unlike FoxO3a deficiency, FoxJ1 deficiency appears to be much more severe, affecting a different spectrum of organs and skewing toward cytokine production by type I Th (Th1)(113). Moreover, recent reports show that FoxQ1 promotes natural killer cell function(140). FoxP1 regulates tissue macrophage differentiation(113). FoxD2 modulates T cell activation by

fine-tuning sensitivity to cAMP(113). Thus it is seen that numerous Fox proteins have numerous roles in regulating immunological activity as summarized in Figure 2.

9. CONCLUSION

Mammalian FOX transcription factors have increasingly become recognized as important targets for disorders of the cardiovascular system and in the immunoregulation of cardiovascular disease pathogenesis. Knowledge of FOX transcription factors will continue to lay the foundation for the successful translation of these transcription factors into novel and robust clinical therapies(141).

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Abbreviations: 5-fluorouracil (5-FU), adherens junction (AJ), protein kinase B (Akt), angiotensin II (Ang II), bone morphogenetic protein (BMP), BMP endothelial cell precursor-derived regulator (BMPER), betacellulin (BTC), CCAAT/enhancer binding protein (C/EBP), CREB binding protein (CBP), CBP-interacting transactivator (CITED), CD36 and LIMPII analogous-1 (CLA-1), cytotoxic T lymphocyte antigen 4 (CTLA-4), cysteine-rich protein 61 (CYR61), Delta-like 4 (Dll4), diabetes mellitus (DM), endothelial nitric oxide synthase (eNOS), endothelial progenitor cell (EPC), extracellular signal-regulated kinase (ERK), endothelial-specific molecule (ESM), E26 transforming-specific sequence (ETS), CD95 (Fas), Fas ligand (FasL), forkhead in rhabdomyosarcoma (FKHR), FKHR like protein 1 (FKHRL1), FLICE-inhibitory protein (FLIP), forkhead (Fox), glycogen synthase kinase (GSK), hydroxy-methylglutaryl coenzyme A reductase (HCR), homocysteine (Hcy), hepatocyte growth factory (HGF), hyperhomocysteinemia (HHcy), hematopoietic stem cell (HSC), human homologue of scavenger receptor of the BI class (hSR-BI), insulin-like growth factor-1 (IGF1), IGF1 receptor (IGF1R), NF-κB inhibitor (IκB), interleukin (IL), immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX), insulin receptor (IR), insuling receptor substrate (IRS), low density lipoprotein (LDL), lipopolysaccharide (LPS), monocyte chemotactic protein (MCP), mitogen-activated protein kinase kinase (MEK), mixed-lineage leukemia (MLL), matrix metalloproteinase (MMP), manganese-superoxide dismutase (MnSOD), mammalian target of rapamycin (mTOR), nuclear factor of activated T cell (NFAT), nuclear factor-κB (NF-κB), ribosomal p70 S6 kinase (p70 S6K), plasminogen activator inhibitor-1 (PAI-1) phosphatidylinositol 3-kinase (PI3K), protein kinase C (PKC), protein phosphatase 2 (PP2), peroxisome proliferator-activated receptor (PPAR), peroxovanadate (PV), reactive oxygen species (ROS), severe combined immunodeficiency (SCID), small interfering RNA (siRNA), mammalian homologue of silent mating type information regulation-2 (SIRT1), signal transducers and activators of transcription (STAT), T cell factor (Tcf), transforming growth factor (TGF), T helper cell (Th), tight junction (TJ), constitutively active mutant of FOXO3a (TM-FOXO3a), tumor necrosis factor (TNF), regulatory T cell (Treg), tuberous sclerosis protein 2 (TSC2), vascular endothelial cell adhesion molecule (VCAM), vascular endothelial growth factor (VEGF), vascular smooth muscle cell (VSMC)

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