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FOXOs in Cancer Immunity: Knowns and Unknowns

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Abstract

In the tumor microenvironment (TME), cancer cells, stromal cells, and immune cells, along with their extracellular factors, have profound effects on either promoting or repressing anticancer immunity. Accumulating evidence has shown the paradoxical intrinsic role of the Forkhead box O (FOXO) family of transcription factors in cancer, which can act as a tumor repressor while also maintaining cancer stem cells. FOXOs also regulate cancer immunity. FOXOs promote antitumor activity through negatively regulating the expression of immunosuppressive proteins, such as programmed death 1 ligand 1 (PD-L1) and vascular endothelial growth factor (VEGF) in tumor cells or stromal cells, which can shape an immunotolerant state in the TME. FOXOs also intrinsically control the anti-tumor immune response as well as the homeostasis and development of immune cells, including T cells, B cells, natural killer (NK) cells, macrophages, and dendritic cells. As a cancer repressor, reviving the activity of Foxo1 forces tumor-infiltrating activated regulatory T (T_{reg}) cells to egress from tumor tissues. As a promoter of cancer development, Foxo3 and Foxo1 negatively regulate cytotoxicity of both CD8⁺ T cells and NK cells against tumor cells. In this review, we focus on the complex role of FOXOs in regulating cancer immunity due to the various roles that they play in cancer cells, stromal cells, and immune cells. We also speculate on some possible additional roles of FOXOs in cancer immunity based on findings regarding FOXOs in non-cancer settings, such as infectious disease.

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Keywords

FOXOs; cancer immunity; anti-tumor immune response; tumor microenvironment

1. Introduction

It has been widely accepted that a series of stepwise events must be initiated and expanded iteratively for generating a productive anticancer immune response that effectively kills cancer cells, a process which is termed the cancer-immunity cycle [1]. Briefly, neoantigens released during oncogenesis are either passively transported into lymph or captured by dendritic cells (DCs) in regional lymph nodes. Next, DCs present tumor-derived antigens on major histocompatibility complex (MHC) molecules and activate antigen-specific CD4⁺ T cells and CD8⁺ T cells in tumor-draining lymph nodes. Newly activated effector T cells leave the lymph node, circulate throughout the body, extravasate through blood vessels, and migrate into the tumor bed where they can target and kill tumor cells displaying their cognate antigen. Then, cancer cell death releases additional tumor antigens that in turn initiate another revolution of the cancer-immunity cycle. Under ideal circumstances, the cancer-immunity cycle amplifies and propagates, leading to the eradication of malignant cells by cytotoxic immune cells, establishment of tumor-specific immunological memory and further prevention of tumor progression [1, 2]. This means that during early neoplasia, cancer is visible to the immune system, i.e., immunogenic [3]. However, the interaction between the immune system and cancer cells is far more complex, continuous, dynamic, and is continuously evolving from the initial establishment of a cancer cell to the development of metastatic disease that is dependent on immune evasion [4]. The immune system not only protects the host against developing tumors but also helps to shape tumor immunogenicity, which is termed cancer immunoediting, that helps to establish tumor immune evasion and resistance [3, 5]. Furthermore, in the tumor microenvironment (TME), the non-malignancy cells and non-immune cells along with their secreted proteins have profound effects on both promoting and repressing anti-cancer immunity [2].

The forkhead box (FOX) family of transcription factors consists of 19 sub-families that share a highly conserved DNA-binding domain of approximately 110 amino acids, the forkhead box domain (also known as the winged-helix domain) [6]. Within this family, the conserved O subgroup family consists of four members: FOXO1 (also known as FKHR), FOXO3 (also known as FKHL1), FOXO4 (also known as AFX1), and FOXO6 in mammals. FOXO1, 3, and 4 are ubiquitously expressed at various levels depending on tissue type, whereas the expression of FOXO6 is restricted to the central nervous system [7]. Although the FOXO-mediated signal-transduction pathways are conserved evolutionarily in most species, they seem to have been co-opted in differentiated tissues for a variety of specialized functions [8]. In mammals, FOXOs are regulated by growth factors, oxidative stress, or nutrient deprivation, and transcriptionally program cellular differentiation, survival, cell-cycle arrest, metabolism, stress resistance, and tumor suppression [9]. The activity and subcellular localization of FOXO transcription factors are regulated in either a transcriptional or posttranscriptional manner [7, 10]. The role of FOXO transcription factors in oncogenesis and cancer therapy has been summarized recently in other reviews [6, 11]. A

great deal of evidence has also revealed that the FOXO family of transcription factors plays a profound role in regulating the development and function of immune cells [12–14], in addition to its immunoregulatory effect through modulating non-malignancy cells and non-immune cells in the TME. In the current review, we will discuss the potential role of FOXOs in regulating cancer immunity from the perspective of cancer cells, immune cells, and other types of cells within the TME.

2. Immune regulation by FOXOs through modulating tumor cells and stromal cells

2.1 Tumor cell-intrinsic factors regulated by FOXOs, and their contribution to the immune response

Tumor cell-intrinsic factors can activate or repress certain genes and pathways, such as mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), Wntless and INT-1 (WNT), and Interferon (IFN), allowing tumor cells to repress immune cell infiltration or function within the TME. Through these signaling pathways, tumor cells secrete immunosuppressive proteins, such as vascular endothelial growth factor (VEGF), transforming growth factor β (TGF- β), indoleamine-2, 3-deoxygenase (IDO), and arginase 1 (ARG1) to establish an immunotolerant state, thus allowing tumor cell survival, proliferation, or metastasis. These pathways may exist at the initial stages of tumorigenesis or may become involved later, for example, during the course of immune therapy [4]. Neoplastic cell-derived factors, such as interleukin (IL)-6 or granulocyte-macrophage colony-stimulating factor (GM-CSF), change the steady-state activity of mature myeloid cells in the TME, which in turn increases recruitment and proliferation of immature myeloid cells, an occurrence that would be atypical under normal physiological conditions, leading to repression of the antitumor immune response [3, 15].

In addition to the aforementioned secreted factors, tumor cells can also regulate the immune response through modulating expression of surface markers. For instance, programmed death 1 ligand 1 (PD-L1), which is constitutively expressed on the surface of tumor cells, actively inhibits T cell activity through binding to its surface receptor, programmed death 1 (PD-1) [16]. Mechanisms ascribed to constitutive expression of PD-L1 include its genetic amplification, phosphatase and tensin homolog (PTEN) deletions or PI3K and/or protein kinase B (PKB or AKT) mutations, epidermal growth factor receptor (EGFR) mutations, MYC overexpression, cyclin-dependent kinase (CDK) 5 disruption, and an increase in PD-L1 transcripts stabilized by truncation of the 3'- untranslated regions (UTRs) of this gene [4]. As a tumor suppressor, FOXO participates in regulating the signaling pathways upstream of PD-L1 via a transcriptional or post-translational manner. For example, FOXO acts downstream of PI3K/AKT signaling and can also suppress MYC oncogenic activity through both downregulating MYC expression and promoting expression of its antagonist, Mxi1 splicing variant strong repressor- α (Mxi-SR α) in colon and renal cancer cells [17, 18]. These studies suggest that FOXO may indirectly upregulate the T cell response by inhibiting PD-L1 expression in cancer cells.

VEGFs, a family of soluble factors including VEGF-A (also known as VEGF), VEGF-C (also known as Flt4-L), and VEGF-D (also known as FIGF), play a critical role in promoting vascularization and lymphangiogenesis in the TME, thus mediating the metastatic spread of tumor cells. VEGF-A promotes blood endothelial cell (BEC) proliferation and angiogenesis, whereas VEGF-C and VEGF-D induce proliferation and activation of lymphatic endothelial cells (LECs) and lymphangiogenesis [19]. Notably, VEGF-A promotes the proliferation of immunosuppressive cells, and represses the recruitment of T cells into tumors, while also promoting T cell exhaustion. VEGF-targeting therapies have been proven to enhance T helper (Th) type 1 cell responses and increase T cell infiltration in tumors [20]. For example, oncogenic signaling activated by the MAPK pathway allows melanoma cells to secrete VEGF proteins, which inhibit T cell function and recruitment to local tumor tissue. The BRAF inhibitor, PLX4720, increases tumor infiltration of adoptively transferred T cells via inhibiting VEGF-A production [21]. One study reveals that FOXO3 can repress VEGF expression in breast cancer cells via direct binding to the VEGF-A promoter [22]. There is also some clinical evidence supporting the role of FOXOs in repressing VEGF-A expression together with reducing vascularization. A higher level of the inactive phosphorylated form of FOXO1 (pFOXO1), which is of clinical significance in the setting of gastric cancer, was positively correlated with the expression of VEGF-A and other angiogenesis-related molecules (e.g., hypoxia inducible factor-1 α) as well as CD34-immunopositive microvessel areas in carcinoma tissue of gastric cancer patients [23]. Coupling with the evidence that treatment with the anti-angiogenic drug, sunitinib, is correlated with improved immune responses in metastatic renal cancer patients [24, 25], it is likely that the role of FOXOs as tumor suppressors is at least partially mediated by their capacity to downregulate VEGF-A, and in turn to boost immune responses to tumor cells. Paradoxically, in prostate cancer cells, FOXO1 was found to promote transcription of VEGF-C, a factor which is implicated in lymph node metastasis of prostate cancer [26].

WNT signaling regulates many aspects of development and tissue homeostasis, and aberrant WNT signaling can lead to cancer. High levels of WNT/ β -catenin activation are intrinsic to melanoma, and result in T cell exclusion and repression of T cell-mediated antitumor immunity via repression of melanoma cell expression of chemokine (C-C motif) ligand 4 (CCL4), a chemokine attractant of CD103⁺ DCs that can activate T cells in the TME. This is believed to be the main mechanism for resistance to anti-PD-L1 or anti-cytotoxic T lymphocyte antigen 4 (CTLA4) monoclonal antibody therapy in patients with melanoma [27]. Several lines of evidence support a role for FOXOs as repressors of the WNT/ β -catenin signaling pathway: Firstly, FOXO1 inhibits the Wnt/ β -catenin pathway during osteosarcoma oncogenesis [28]; Secondly, FOXOs compete with T cell factor (TCF) for interaction with β -catenin, thereby inhibiting β -catenin-mediated gene transcription under oxidative stress [29–31]; Thirdly, FOXO3 can repress β -catenin expression through transactivating the expression of microRNA-34b/c that targets the UTRs of beta-catenin [30].

Together, all evidence presented above suggests that FOXOs may foster the anti-tumor immune response in a cancer cell-intrinsic manner through its role as a negative regulator of several immunosuppressive factors, such as PD-L1 and VEGF, as well as a positive regulator of chemokine attractants, such as CCL-4 (Figure 1).

2.2. FOXOs in stromal cells of the TME impacts the immune response

In the TME, there is a complex milieu of non-malignant cells, including stromal cells, myeloid cells, and lymphoid cells along with their secreted extracellular matrix and inflammatory mediators, which together interact closely with tumor cells. This affects tumor growth, survival, invasion and metastatic dissemination, and can impact responsiveness to therapeutics in diverse ways [2, 3]. Stromal cells represent an integral component of solid tumors, comprising BECs, LECs, mesenchymal stem cells (MSCs) and their differentiated progeny, cancer-associated fibroblasts (CAFs), and pericytes [2, 15]. The dynamic and mutualistic interaction between stromal cells and the surrounding tumor cells can promote the initiation, progression, and metastasis of solid tumors. In addition, stromal cells can also participate in shaping anti-tumor immunity and responsiveness to immunotherapy [2, 15].

2.2.1 Endothelial cells—BEC proliferation is responsible for angiogenesis while LEC proliferation and activation is implicated in lymphangiogenesis during tissue vascularization [2, 19]. In the process of neoplastic progression, a sustained increase in tissue vascularization, including angiogenesis and lymphangiogenesis, is a common phenomenon that contributes to metastatic dissemination of tumor cells. A growing body of evidence suggests that endothelial cells are crucial regulators of the host's immune response to cancer [2]. For example, treatment with anti-angiogenic drugs in the clinic leads to a reduction in vascularization and, interestingly, is also correlated with a reduction in the number of intratumoral immunosuppressive populations, namely regulatory T cells (T_{reg} cells) and myeloid-derived suppressor cells (MDSCs), which consequently results in an improved anti-tumor immune response [24, 25].

FOXOs have been shown to have an endothelial cell-intrinsic role in managing angiogenesis, vascular stabilization, endothelial cell specification during postnatal neovascularization, ischemic muscle [32–35], and physical exercise [36]. Foxo1 and 3 inhibit endothelial cell migration and tube formation in mature endothelial cells, and mechanistic studies have revealed this occurs via their specific regulation of a non-redundant but overlapping set of angiogenesis- and vascular remodeling-related genes [32]. A recent study has also revealed that FOXO1 acts as a gatekeeper of endothelial quiescence via suppression of MYC-mediated anabolic metabolism. Accordingly, an endothelial cell-specific knockout of Foxo1 in mice led to enhanced endothelial cell proliferation that interferes with coordinated sprouting, which in turn causes hyperplasia and vessel enlargement. Conversely, Foxo1 overexpression restrains vascular expansion and results in vessel thinning and hypobranched [37]. Endothelial cell expression of FOXO1 is also critical for endothelial cells to respond properly to high dose VEGF-A; in particular, murine endothelial cells defective in Foxo1 showed a markedly abnormal morphological response upon exposure to exogenous VEGF-A [38]. In addition, FOXO1 also promotes the differentiation of human embryonic stem cells toward the endothelial lineage, but represses the proliferation and tube formation of human embryonic stem cell-derived endothelial cells [39]. Likewise, there is also evidence to suggest a regulatory role for FOXO3 in endothelial cells. Namely, FOXO3 has been shown to repress endothelial progenitor cell proliferation via a mechanism involving upregulation of p27 (kip1) and downregulation of CDK2, cyclin D1, and proliferating cell nuclear antigen (PCNA) [40].

Thus, FOXOs regulate endothelial cell homeostasis (as described immediately above) and, as stated in the Section 2.1, FOXOs can also negatively regulate tumor cell production of secreted factors, including VEGF-A, in a cell-intrinsic manner [21–23]. This implies that FOXOs can repress tumor angiogenesis by acting on both cancer cells and endothelial cells, which in turn influences the immune response in the TME (Figure 1).

2.2.2 MSCs and CAFs—In addition to endothelial cells, FOXOs impact other stromal cell compartments, including MSCs and CAFs. MSCs are multipotent stromal cells that can be recruited to tumors, where they differentiate into fibroblasts and pericytes. FOXOs are reportedly involved in MSC terminal fate decisions via their role in governing defense mechanisms against oxidative stress. FOXOs, along with peroxisome proliferator-activated receptor- γ (PPAR- γ), CCAAT/enhancer-binding protein (C/EBP), bone morphogenetic protein (BMP), and insulin growth factor (IGF) signaling, promote adipogenesis but repress osteogenesis of MSCs [41].

CAFs have been shown to promote cancer cell growth and metastasis via nurturing tumor cells and cancer stem cells through secretion of several growth factors, such as hepatocyte growth factor (HGF), fibroblast growth factor (FGF), IGF 1 and 2, as well as TGF- β [2]. CAF accumulation and activation in the TME are correlated with poor prognosis in several cancers [42, 43]. Previous studies have revealed that FOXOs, including FOXO1 and 3, can repress proliferation and promote cellular senescence of human fibroblasts via regulating the expression of platelet-derived growth factor (PDGF) [44–46]. This indicates that FOXOs might act as a repressor of stromal cell- or CAF-mediated cancer immune evasion in the TME. However, this theory remains to be validated using MSC- or fibroblast-specific Cre mice to delete FOXOs in MSCs or fibroblasts, respectively, in the context of cancer.

Collectively, the evidence discussed here in Section 2 of this review indicates that integrative actions of FOXOs in cancer cells, endothelial cells, and fibroblasts can shape anticancer immunity extrinsically, which could be critical for constraining the progression of cancer (Figure 1).

3. Effect of FOXO signaling in immune cells

3.1 FOXO-mediated regulation of the myeloid compartment in the TME

Myeloid cells, including macrophages, DCs, mast cells, monocytes, and granulocytes have multiple homeostatic functions in an evolving neoplasm, such as antigen capture, tissue repair, and regulation of effector functions [3]. The plasticity and communication between the myeloid compartment and other immune cells or stromal cells are pivotal for tumor formation and progression [3, 47].

3.1.1 DCs—DCs can activate both the innate and adaptive immune responses through secreting cytokines or acting as antigen-presenting cells (APCs) [48]. Compared to other APCs, DCs are perhaps the most effective at inducing an antigen-specific T-cell response [3, 49]. Soluble cancer antigens are transported to lymph nodes via lymphatic vessels, where they are captured by lymph node-resident DCs, while tissue-resident DCs within the TME capture antigens at the site of the tumor. Both lymph node- and tissue-resident DCs can

present tumor antigens to antigen-specific T cells, such as CD8⁺ T cells, CD4⁺ T cells, and natural killer T (NKT) cells. When tumor antigen presentation occurs in conjunction with engagement of costimulatory molecules (CD80, CD70, 4-1BB) and DC-derived interleukin (IL)-12, type I interferon, and IL-15, naïve CD8⁺ T cells differentiate into cytotoxic T lymphocytes (CTLs), whereas naïve CD4⁺ T cells either differentiate into one of the helper T cell subsets, each with a unique cytokine production repertoire, or into FoxP3⁺ T_{reg} cells, which possess a repressive role in regulating CTL activity [50]. Previous studies have shown that intratumoral DCs expressing CD103 in mice, or CD141 in humans, drive activation of intratumoral CD8⁺ T cells. C-C chemokine receptor 7 (CCR7) is expressed on the surface of these DCs, mediating their trafficking to lymph nodes to activate CD8⁺ T cells there. In patients with melanoma, high levels of CCR7 are positively correlated with greater T cell infiltration into the tumor and better survival [51].

Previous studies showed that Foxo1 is generally found in its phosphorylated inactive form in DCs, which ensures their survival and normal proliferation, especially when DCs are stimulated by CCR7 ligand or immunological synapse formation with T cells [52, 53]. Knockdown of Foxo1 in serum-free conditions leads to a significantly lower percentage of apoptotic DCs [53]. The transcriptional activity of FOXOs also plays a fundamental role in maintaining normal homing of DCs to lymph nodes. In particular, Foxo1 promotes transcript expression of CCR7 and intercellular adhesion molecule-1 (ICAM-1), both of which are critical for DC-mediated stimulation of T and B lymphocytes in response to bacterial infection [54–56]. In contrast, Foxo3 suppresses DC production of key inflammatory cytokines, such as IL-6 and TNF, constraining CD4⁺ and CD8⁺ T cell population expansion after viral infection [57]. Foxo3-deficient dendritic cells produce higher levels of IL-6, which sustains T cell viability and expansion in response to lymphocytic choriomeningitis virus (LCMV) infection [57]. Together, this evidence indicates that the FOXO family plays complex roles in regulating the survival, trafficking, and activation of DCs (Figure 2). It would be interesting to know whether a similar phenomenon may also occur during the anti-tumor response; however, thus far, this topic has remained largely unexplored.

3.1.2 Macrophages—Macrophages account for a major component of the leukocyte infiltration in most tumors [58]. Several studies in humans have revealed that the presence of intratumoral macrophages is correlated with increased disease aggressiveness and poorer outcome, findings which can be interpreted as evidence in favor of macrophages as potential targets of cancer immunotherapy. Within tumors, macrophages can serve dual roles. Their influence can be supportive or inhibitory, depending on their polarization: classically activated M1-like macrophages can boost the anti-tumor responses of T cells, destroy tumor stroma, and normalize tumor vasculature to promote effective anti-tumor immunity [59]; however, M2-like macrophages, generally known as tumor-associated macrophages (TAMs), usually have tissue-reparative activity, which supports tissue remodeling while suppressing the type I immune response [59]. M2-like macrophages can promote tumor progression by inhibiting anti-tumor T cell responses and secreting cytokines (e.g., TGF-β) and amino acid-degrading enzymes (e.g., ARG1 and IDO), resulting in T_{reg} cell accumulation in the TME [59, 60]. With this in mind, ‘re-educating’ TAMs to become M1-like macrophages or

directly targeting M2- like TAMs have both been considered as potentially promising therapeutic strategies.

During classical activation following lipopolysaccharide (LPS) treatment, M2-like macrophages showed increased expression of Foxo1 compared to that in M1-like macrophages. Furthermore, Foxo1 tends to bind to the promoter of IL-10 in M2-like macrophages, but not in M1-like macrophages. After challenge with LPS, mice with macrophage-specific deletion of Foxo1 (achieved through the use of lysozyme 2 (LysM)-Cre mice) showed a reduction in M2-like cells and an increase in M1-like cells. In particular, this occurred due to a significant reduction in the expression of M2-like macrophage markers (IL- 10, Arg1, Fizz1, and interleukin 13 receptor alpha 1 (IL-13Ra1)), while expression of M1- like macrophage markers (inducible nitric oxide synthase 2 (iNOS), IL-12a, IL-12b, and Ccr2) was increased compared with wild-type mice [61] (Figure 2). However, whether FOXOs activity differs among human macrophage subsets, and whether FOXOs promote the development of M2-like macrophages in patients with cancer remains to be revealed.

3.2. Regulation of the lymphoid compartment of the TME by FOXOs

The lymphoid compartment in tumors includes CD4⁺ T cells, CD8⁺ T cells, NKT cells, B cells, and NK cells. The functional activity of these lymphocyte subsets depends upon the expression of restriction elements, including peptide-MHC complexes (pMHC; for T cells), the MHC class I molecule (for NK cells), or immunogenic surface proteins (for B cell products, i.e., antibodies) that can be recognized in a specific manner [3]. Additionally, lymphoid cells can be induced to secrete several types of cytokines.

3.2.1 T cells—T cells, especially cytotoxic CD8⁺ T cells and CD4⁺ T_H1 cells, are the principal weapons of the immune system against cancer. Thymocytes undergo a series of selection processes before becoming mature CD4⁺ or CD8⁺ T cells. Mature CD4⁺ or CD8⁺ T cells express several trafficking molecules [e.g., sphingosine 1-phosphate receptor 1 (S1P1), CCR7, and CD62L], which endow them with the capacity to migrate from the thymus into peripheral lymphoid organs. In peripheral lymphoid organs, IL-7 plays a pivotal role in maintaining naïve T cell survival and homeostatic proliferation [14]. Well-orchestrated T cell trafficking, survival, and homeostatic proliferation is critical for T cell homeostasis and activation upon antigen presentation. Recent studies revealed that Foxo1, which plays a more prominent role than Foxo3, is critical for controlling T cell trafficking via its ability to promote the expression of trafficking molecules (specifically S1P1, CCR7, and CD62L) at the transcriptional level, and also maintaining survival and homeostasis via directly promoting expression of the IL-7 receptor (IL-7R, also known as CD127) [14, 62–65] (Figure 3, Immune tissues). In contrast, an *in vitro* study found that phosphorylation, i.e., inactivation, of Foxo3 plays a positive role in IL-2-mediated T cell survival. In particular, promoting FOXO3 transcriptional activity via an inducible gene regulation approach is sufficient to induce T cell apoptosis that mimics the effects of IL-2 withdrawal via direct targeting of the cdk inhibitor, p27Kip1, and the proapoptotic Bcl-2 family member, Bim [66].

3.2.1.1 T helper cells: FOXOs play a critical role in the differentiation of naïve CD4⁺ T cells into effector or memory T cells upon antigen presentation. For example, when compared to unstimulated control cultures, *in vitro* stimulation of Foxo1-deficient naïve T cells with phorbol 12- myristate 13-acetate (PMA) and ionomycin resulted in T cell production of large quantities of IFN- γ , IL-4 and IL-17, but not IL-2, indicating an increase in T cell differentiation which was biased toward T_H effector cells. Interestingly, upon stimulation through CD3 and CD28 in the presence of with TGF- β , naïve CD4⁺ T cells will differentiate into T_{reg} cells; however, when it occurs in the absence of Foxo1, the very same stimulation will instead misdirect these cells to acquire a T-bet⁺ IFN- γ ⁺ T_H1-like phenotype [65] (Figure 3, Immune tissues). *Foxo3* mRNA is highly expressed by naïve T_H cells and diminishes rapidly upon anti-CD3 and/or IL-2 stimulation. Compared to their wild-type counterparts, naïve T_H cells with Foxo3-deficiency produced higher levels of T_H1 cytokines, such as IL-2 and IFN- γ , or had enhanced expression of T_H2 cytokines, such as IL-4, IL-5, IL-6 and IL-10, in response to T_H1 or T_H2-polarizing conditions, respectively [67] (Figure 3, Immune tissues). T_H1 effector cells can promote the cytotoxic activity of CD8⁺ T cells and macrophages, and induce upregulation of antigen processing and expression of MHC I and II molecules in professional APCs (i.e., macrophages and DCs) [3, 68]. T_H1 cells are also necessary for induction of the NK cell-mediated antitumor response [68].

3.2.1.2 T_{reg} cells: T_{reg} cells, which are indispensable for maintaining dominant self-tolerance and immune homeostasis, are defined by expression of the transcription factor forkhead box P3 (Foxp3) and surface expression of CD25 [69]. T_{reg} cells can also repress antitumor immune activity via cell-to-cell contact-dependent or -independent mechanisms, and thus promote tumor progression. Deletion of T_{reg} cells or disruption of their differentiation can promote strong, systemic antitumor T cell reactivity and immunosurveillance against cancer cells [70]. The TME is favorable for inducing and maintaining T_{reg} cell identity by both *de novo* generation from CD25⁻ T cells (inducible T_{reg}, iT_{reg}) that are converted from naïve CD4⁺ T cells found in the periphery, and also by promoting recruitment of thymus-derived naïve (n)T_{reg} cells to the tumor site [71]. T_{reg} cells often act to impair activation, survival, and expansion of antitumor T cells via expression of the immune-inhibitory receptor CTLA4 and production of immunosuppressive cytokines, such as TGF- β and IL-10 [72].

FOXOs play a critical role in controlling the generation of both nT_{reg} and iT_{reg} cells [14]. An initial study using Foxo1 and Foxo3 double knockout T cells in a CD4-Cre mouse model showed a reduction in the number of nT_{reg} cells in the thymus and spleen of 3-week-old mice [73]. Kerdales et al. provided additional evidence indicating that T cell-specific deletion of Foxo1 severely curtailed the development of nT_{reg} cells [65]. Foxo proteins not only bind to the Foxp3 promoter directly and control its transcription in thymus, which could trigger robust Foxp3 expression [73], but also repress expression of T-bet, a transcription factor that is critical for T_H1 cell differentiation [65]. In addition, FOXOs are also critical for the generation of iT_{reg} cells in response to T cell receptor (TCR), IL-2, and TGF- β stimulation [73, 74], which occurs downstream of PI3K-AKT-mTOR signaling [75] (Figure 3, Immune tissues).

Foxo proteins are also important for maintaining T_{reg} cell function. T cell-specific deletion of Foxo1 using a CD4-Cre mouse model revealed that Foxo1 is critical for controlling T cell specification, as Foxo1-deficient CD4⁺ T cells showed reduced Ctla-4 expression and were unable to repress T-bet expression in response to *in vivo* or *in vitro* TGF- β treatment [65]. Further studies using Foxp3-Cre mice demonstrated that Foxo1 acts beyond the T_{reg} cell commitment stage, also controlling T_{reg} cell homeostasis and function [76]. Foxo1 resides in the nucleus during quiescence, but becomes phosphorylated by Akt and translocates to the cytosol upon TCR stimulation in both T_{reg} cells and conventional CD4⁺ T cells. However, compared to conventional T cells, T_{reg} cells express higher levels of Foxo1, display lower levels of phosphorylated Akt and Foxo1, and thus exhibit a reduction in nuclear exclusion of Foxo1 following stimulation through the TCR. T_{reg} cell-specific deletion of Foxo1 using Foxp3-Cre mice leads to a reduction in Ctla-4 expression and an increase in IFN- γ production, leading to a fatal inflammatory disorder [76] (Figure 3, Immune tissues).

However, a recent study using an *in vivo* B16 melanoma cell model revealed an unexpected role for Foxo1 in repressing immune tolerance mediated by tumor-infiltrating activated T_{reg} cells [77]. In particular, compared to resting T_{reg} cells in secondary lymphoid organs, tumor-infiltrating activated T_{reg} cells possess higher levels of phosphorylated Akt and Foxo1, together with lower expression of Foxo1-activated genes such as CCR7 and Bim, which are thought to potentially be involved in T_{reg} cell homing to lymphoid organs [77]. Expression of a constitutively activated form of Foxo1, achieved through the use of a Foxo1 mutant that is refractory to Akt-triggered phosphorylation, specifically within the T_{reg} subset reversed the downregulation of lymphoid organ homing molecules, such as CD62L, thus promoting egress of tumor-infiltrating activated T_{reg} cells from tumor tissue, resulting in restored activation of effector CD8⁺ T cells, which were able to repress tumor growth without inflicting autoimmunity [77] (Figure 3, Intratumoral Stroma).

3.2.1.3 CD8⁺ T cells: CD8⁺ T cells, especially CD8⁺ tumor-infiltrating lymphocytes (TILs), play a major role in the adaptive immune responses against tumors [78]. The frequency of CD8⁺ TILs has been shown to positively correlate with cancer-free survival in patients with melanoma, lung, breast, brain, and colorectal cancer [78–82]. In the context of antitumor immunity, CD8⁺ T cells can be classified into at least six subsets, including effector T cells, memory T cells, exhausted T cells, regulatory T cells, anergic T cells, and senescent T cells; however, it should be noted that, at present, there is no universally accepted classification system for CD8⁺ T cells. Upon TCR engagement with antigen and co-stimulation by APCs, naïve CD8⁺ T cells differentiate into effector T cells (T_{EFF}), which show high capacity for cytotoxicity and expression of IFN- γ , tumor necrosis factor (TNF- α), granzymes, and perforin following activation. CD8⁺ T_{EFF} cells represent the majority of TILs in well-controlled tumors, and are believed to be responsible for positive clinical responses following adoptive T cell transfer [83, 84]. During the resolution stage of several models of acute infection, T_{EFF} cells undergo a rapid, apoptosis-induced contractile phase, and a subset of antigen-specific CD8⁺ T cells become memory CD8⁺ T cells following antigen elimination [85–87]. Based on their expression of the surface markers CD62L and CCR7, memory CD8⁺ T cells can be divided into two subsets: effector memory (T_{EM}) and central memory (T_{CM}). T_{EM} cells, which are CCR7 negative, with intermediate levels to no

expression of CD62L, can migrate to inflamed tissues and execute immediate effector function upon secondary stimulation. T_{CM} cells, which are CCR7 positive and CD62L high, can home to secondary lymphoid organs and efficiently stimulate dendritic cells, but lack capacity for immediate effector function [78, 88]. Upon secondary antigen stimulation, T_{CM} cells differentiate into CCR7⁻ effector cells less rapidly compared to T_{EM} cells; however, T_{CM} cells have higher potential for proliferation, greater self-renewal capacity, and are more prolific producers of IL-2 [89]. Recent studies found that T_{EM} and T_{CM} subsets both exist in breast and colorectal cancer patients [90–92]. Upon adoptive transfer, both aforementioned subsets undergo further development *in vivo* and give rise to progeny capable of differentiating into T_{EFF} cells, responsible for maintaining effector function and contributing to tumor regression [93, 94].

In CD8⁺ T cells, FOXOs play a defining role in the regulation of effector vs memory CD8⁺ T cell differentiation. Upon stimulation with antigens, Foxo1 becomes inactivated in effector CD8⁺ T cells downstream of the TCR/PI3K/AKT axis [95], which releases Foxo1-mediated inhibition of T-bet, allowing these CD8⁺ T cells to perform their typical effector functions [96]. Indeed, deletion of Foxo1 has been shown to promote CD8⁺ T cell effector functions [96]. Deletion of Foxo3 enhances the accumulation of primary antigen-specific CD8⁺ T cells primarily by inhibiting their apoptosis while rate of cell division remains unaffected [97, 98], thus promoting a more effective immune response against infections [98, 99]. Macrophage stimulating 1 (Mst1) and Macrophage stimulating 2 (Mst2) are mammalian hippo kinases that inhibit cell proliferation while promoting apoptosis. In a recent study, the role of Mst1 in suppressing the expansion of tumor-eradicating CD8⁺ cytotoxic T cells was found to be mediated by Foxo3 activation. Accordingly, decreased levels of Foxo3 expression in naïve Mst1^{-/-} CD8⁺ T cells made these cells more prone to differentiate into cytotoxic CD8⁺ T cells [100] (Figure 3, Immune tissues).

However, FOXOs also play a critical role in the differentiation of memory CD8⁺ T cells. In particular, due to its role in induction of TCF7 and Eomes expression, Foxo1 is essential for generating the precursors of memory CD8⁺ T cells [96, 101]. Deficiency of Foxo1 in T cells impairs the differentiation of memory CD8⁺ T cells, and abolishes recall responses upon secondary infection [96, 101]. In an irradiation-induced lymphopenic mouse model into which orthologous T cells were transplanted, Foxo1, acting downstream of IL-15 signaling, promoted the development of CD8⁺ memory T cells [102]. Despite the increased expansion of Foxo3-deficient effector T cells, the precursors of memory T cells also accumulate, resulting in an increased quantity of CD8⁺ memory T cells. However, this increase in CD8⁺ memory T cells does not trigger a stronger recall response, suggesting that Foxo3 may also function in regulating the recall responses of memory T cells [97]. This phenomenon also exists in murine tumor tissue after treatment with a cancer vaccine. For an example, following vaccination with sarcoma antigen (SarAg), along with a global immunomodulator, neem leaf glycoprotein (NLGP), the antigen-specific CD8⁺ T memory cells generated in lymph nodes showed reduced levels of Foxo3 phosphorylation in the cytosol together with enhanced expression of Foxo3 target genes, such as CD62L and CCR7. The T_{CM} and T_{EM} cells that were generated were protective, and ultimately sustained disease-free survival following post-vaccination tumor inoculation [103] (Figure 3, Immune tissues).

Exhausted T cells are a population of persistent T cells with low proliferative potential, reduced IL-2 and IFN- γ production, and reduced cytotoxic activity. Exhausted T cells exist in the context of chronic viral infection and also cancer, a phenomenon believed to be attributable to persistent antigen exposure [78, 104]. During the anti-tumor immune response, a number of inhibitory receptors, such as PD-1, CTLA-4, T-cell Ig-and mucin-domain-containing molecule-3 (TIM-3), CD160, the lymphocyte activation gene 3 (LAG-3), and CD244 (2B4), are upregulated on the surface of exhausted CD8⁺ T cells, which acts to prohibit sustained activation while supporting survival of exhausted CD8⁺ T cells in response to persistent tumor antigen exposure [105–110]. PD-1^{hi} exhausted CD8⁺ T cells from patients with melanoma show increased expression of activation markers, and also produce similar level of cytokines (e.g., IFN- γ and TNF- α) in response to *ex vivo* stimulation [111, 112]. The antitumor response of exhausted CD8⁺ T cells can be restored with the use of PD-L1 blocking antibodies [113]. This finding demonstrates that the exhausted phenotype of PD-1^{hi} CD8⁺ T cells may be controlled by external regulatory mechanisms involving PD-1 signaling [113]. Interestingly, a recent study revealed that the transcriptional activity of Foxo1 is essential for promoting expression of PD-1 and Bcl2, which are responsible for CTL adaption and survival during chronic LCMV infection [114]. If this phenomenon also occurs in the TME, the imbalance of PD-1-PD-L1 regulation by FOXOs between CD8⁺ T cells and cancer cells is a critical step in activating CD8⁺ T cells to eradicate tumor cells in patients. In other words, the loss of FOXOs activity leads to increased expression of PD-L1 in cancer cells [17, 18], but simultaneously decreases expression of PD-1 in CD8⁺ T cells in the TME; the former of which inhibits, while the latter promotes activity of cytotoxic CD8⁺ T cells against tumor cells. As Foxo1 also promotes Ctl4 expression in T_{reg} cells [76], it is possible that Foxo1 may also act as an adaptor of exhausted CD8⁺ T cell survival during the process of cancer development (Figure 3).

Because they represent the major subset of cytotoxic T cells, improvement in quantity and function of effector CD8⁺ T cells can enhance anti-tumor responses greatly during immunotherapy [78]. *In vitro* generated T_{CM} cells, which show higher antitumor activity *in vivo* than that of transferred T_{EFF} cells, also show a good capacity for robust expansion and tumor rejection in an *in vivo* murine model of melanoma [93, 94]. Due to the distinct effects of FOXO activity in regulating effector, memory, and also exhausted CD8⁺ T cell subsets, it will be challenging to develop a proper strategy for cancer immunotherapy through modulating the activity or expression of FOXO in T cells. In the case of CD8⁺ T cells, FOXO activity seems to be a negative regulator at the beginning but a positive regulator at the end stages of cancer development (Figure 3, Immune tissues).

3.2.2 NK cells—NK cells are a critical subset of innate lymphoid cells (ILCs) due to their role in performing immunosurveillance of tumors [115]. NK cells are of interest as immunotherapeutic targets [116], because they are readily able to eliminate tumors through cytotoxic molecules, such as perforin and granzyme B, under the control of integral signals from activating and inhibitory ligands. This population also has regulatory functions following stimulation by cytokines such as IL-12, IL-18 and IL-15 [115], as activated NK cells secrete cytokines, particularly IFN- γ , which are critical for engagement in

immunoregulation [115]. An 11-year follow up study confirms that people with NK cells that possess lower levels of natural cytotoxic activity have an increased risk of eventually developing cancer [117].

We and Eric Vivier's group discovered that Foxo1 is a negative regulator of NK cell maturation and acquisition of effector functions. This phenomenon occurs primarily through Foxo1's role in inhibiting expression of T-bet, a transcription factor which is recognized as essential for NK cell effector functions [118]. We found that Foxo1 is downregulated during NK cell development, and NK cell-specific deletion of Foxo1 can promote late-stage maturation, as defined by expression of the surface markers, CD27 and CD11b (Figure 3, Immune tissues). Furthermore, when NK cells become activated by cytokines or tumor cells, Foxo1 tends to be present in its inactive phosphorylated form. Indeed, we found that deletion of Foxo1 enhanced the cytotoxic capacity of NK cells against melanoma cells (Figure 3, Intratumoral Stroma). In a separate study, another group reported that Foxo1-induced autophagy is indispensable for NK cell development and murine cytomegalovirus (MCMV) clearance using an NKp46^{iCre} mouse model that differs from the model as we used [119]. This discrepancy in the reported effects of Foxo1 activity on NK cell activity against tumor cells and virus requires further investigation.

3.2.3 B cells—B cells represent a heterogeneous population with functionally distinct subsets, and B cell-triggered autoimmune responses are associated with several types of tumorigenesis [120]. Specifically, certain autoantibodies from tumors activate acquired humoral immunity, thus acting in a defensive role against naturally occurring tumors [121]. The presence of these autoantibodies have been generally considered as promising biomarkers for quick and early diagnosis of specific tumors [122]. Besides, a few specific subsets of B cells, such as CD11c⁺ or CD20⁺ B cells, may interact with T cells directly in the TME to promote the anti-tumor responses of T cells.

In B cells, Foxo1 and Foxo3 both act as a repressor of proliferation through their role in inducing the expression of cyclin G2, p130 and Kruppel like factor 4 (KLF4), each of which have been implicated in cell cycle arrest [123, 124]. As a result, BCR activation triggers activation of the PI3K/ Bruton tyrosine kinase (BTK)/ B cell linker protein (BLNK)/ phospholipase C gamma 2 (PLCγ2) axis to inhibit Foxo1 expression to ensure continued B cell proliferation [125]. However, FOXOs are also essential for the differentiation of B cells at various stages. For example, deletion of Foxo1 at an early stage blocks the differentiation of pro-B cells due to reduced expression of interleukin 7 receptor-alpha (IL-7Rα), and inactivation of Foxo1 in late pre-B cells inhibits the expression of recombination activating gene (Rag) 1 and Rag2, resulting in arrest at the pre-B cell stage [126]. As for mature B cells, Foxo1 promotes homing to lymph nodes through inducing expression of L-selectin (also named as CD62L) [126] while also promotes normal B cell distribution in the spleen [127]. A global screening approach using chromatin immunoprecipitation combined with deep DNA sequencing (ChIP-Seq) demonstrated that E2A and EBF1 bind to the Foxo1 gene locus and contribute to B cell fate determination [128] (Figure 3, Immune tissues).

Together, the evidence presented here in Section 3 indicates that FOXOs may exhibit contrary effects in regulating anti-tumor immunity in several immune cell subsets in a cell-

intrinsic way. FOXOs can act as a cancer repressor in T_{reg} cells, while acting as a cancer promoter in CD8⁺ T cells and NK cells (Figure 3, Intratumoral Stroma). In addition, FOXOs are critical transcription factors for the homeostasis, trafficking, survival, proliferation, and/or development of many immune cell types, such as T cells, NK cells, B cells, macrophages, and DCs (Figure 3, Immune tissues).

4. Concluding remarks

Although dysfunction of the immune system in the TME has been recognized by virtue of advanced techniques, such as high-resolution single cell sequencing and bioinformatic analysis, the molecular basis for the crosstalk between tumor cells, the TME, and immune cells during the process of cancer development remains to be fully understood. FOXOs, a family of tumor suppressor genes, have been recently identified to mediate the bidirectional communication between tumor cells and the immune system. Genes directly or indirectly targeted by FOXOs in each cell subset are listed in Table 1.

In immune organs of healthy individuals, FOXOs also directly regulate the homeostasis, function, trafficking, survival, proliferation, and/or development of almost every immune cell subset, including macrophages, T cells, NK cells, B cells, and DCs (Figure 1, Immune Tissues). During the process of cancer initiation and progression, FOXO proteins are frequently inactivated by oncogenic signals such as the PI3K pathway and MAPK in cancer cells, which in turn leads to elevated expression of immunosuppressive factors, such as PDL1 and VEGF. FOXOs also promote the expression of anti-cancer immune regulators, such as CCL-4. Furthermore, FOXOs also act in a cell-intrinsic manner to inhibit tumor angiogenesis through regulating the proliferation and sprouting of endothelial cells, and in a cell-extrinsic manner by modulating VEGF production in tumor cells. In addition, FOXOs also restrain human fibroblast proliferation. Together, this evidence indicates that FOXOs play a role in promoting anti-cancer immunity during the process of neoplasia, due to the roles that FOXOs play in tumor cells and stromal cells. However, direct evidence for regulation of the antitumor immune response by FOXOs from the perspective of immune cells is very limited and also complicated. As a cancer repressor, loss of Foxo1 activity in tumor-infiltrating activated T_{reg} cells results in accumulation of this subset within tumor tissue, which strongly represses the anti-cancer immune response. As a tumor promoter, Foxo1 and Foxo3 negatively regulate cytotoxicity of both CD8⁺ T cells and NK cells against cancer cells (Figure 1, Intratumoral Stroma).

Based on these findings, we would like to propose a role for FOXOs as a rheostat, regulating both immune homeostasis and the immune response in cancer immunity. In healthy individuals, higher activity of FOXOs prohibits survival and growth of cancerous cells caused by random mutations, while maintaining normal homeostasis and function of various immune cell subsets. During early neoplasia, the activity of FOXOs is gradually lost in cancer cells, and stromal and T_{reg} cells promote the survival and growth of cancer cells, in addition to establishing an immune-tolerant microenvironment. While this may be true, the activity of FOXOs is also downregulated in cytotoxic immune cells (e.g., CD8⁺ T cells and NK cells) upon encountering cancer cells, and results in activation that is critical for recognizing and rejecting cancer cells. Once the balance between FOXO-mediated

regulation of cancer or stromal cells and activity of cytotoxic immune cells is disturbed, cancer progression will accelerate (Figure 3, Intratumoral stroma).

In the future, more work remains to be completed to further understanding of the network described in Figure 3. It will be interesting to gain a better understanding of each molecule and each cell type that is regulated by FOXOs directly in the setting of cancer patients or tumor models. For instance, it would be of great significance to explore the expression and activity of FOXOs in each immune cell subset within the TME, in addition to peripheral lymphoid organs or tissues of various cancer types using currently available single cell sequencing data [129, 130]. As FOXOs play multi-pronged roles in cancer immunity, and can play a role as either a cancer suppressor or a cancer promoter, it would be of interest to distinguish these roles and identify the predominant roles for each cancer type. Moreover, although FOXOs are intracellular molecules and are difficult to target, indirectly targeting these molecules or directly searching for small compounds to modulate their expression and/or activity would help not only to further understand their roles, but also for developing potential therapeutics. The complex roles of FOXOs may also allow the option of specifically targeting tumor cells or immune cells, or attempting to differentially target both. Thus, further dissecting the role of FOXOs, especially in the setting of anti-tumor immunity, would not only shed light on further understanding cancer initiation and development, but may also help optimize immunotherapeutic strategies for treating or preventing cancers through modulating the activity of FOXOs.

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Abbreviations

2B4	CD244 molecule
AKT or PKB	protein kinase B
APCs	antigen-presenting cells

ARG1	arginase 1
BEC	blood endothelial cell
BLNK	B cell LiNKer protein
BMP	bone morphogenetic protein
BTK	Bruton tyrosine kinase
C/EBP	CCAAT/enhancer-binding protein
CAFs	cancer-associated fibroblasts
CCL4	chemokine (C-C motif) ligand 4
CCR7	C-C chemokine receptor 7
CDK	cyclin-dependent kinase
ChIP-Seq	chromatin immunoprecipitation combined with deep DNA sequencing
CTLA4	cytotoxic T lymphocyte antigen 4
CTLs	cytotoxic T lymphocytes
DC	dendritic cells
EGFR	epidermal growth factor receptor
FGF	fibroblast growth factor
FOXO	Forkhead box O
GM-CSF	granulocyte-macrophage colony-stimulating factor
HGF	hepatocyte growth factor
ICAM-1	intercellular adhesion molecule-1
IDO	indolamine-2, 3-deoxygenase
IFN	Interferon
IGF	insulin growth factor
IL	interleukin
IL-13ra1	interleukin 13 receptor, alpha 1
IL-7R or CD127	IL-7 receptor
IL-7Ra	interleukin 7 receptor-alpha
ILCs	innate lymphoid cells

iNOS	inducible nitric oxide synthase 2
iT_{reg}	inducible T _{reg}
KLF4	Kruppel like factor 4
LAG-3	lymphocyte activation gene 3
LCMV	lymphocytic choriomeningitis virus
LEC	lymphatic endothelial cell
LPS	lipopolysaccharide
LysM	lysozyme 2
MAPK	mitogen-activated protein kinase
MCMV	murine cytomegalovirus
MDSC	myeloid-derived suppressor cells
MHC	major histocompatibility complex
MSCs	mesenchymal stem cells
Mst1	Macrophage stimulating 1
Mst2	Macrophage stimulating 2
Mxi-SRα	Mxi1 splicing variant strong repressor- α
NK	natural killer
NKT	natural killer T
NLGP	neem leaf glycoprotein
nT_{reg}	Naïve T _{reg}
PCNA	proliferating cell nuclear antigen
PD-1	programmed death 1
PDGF	platelet-derived growth facto
PD-L1	Programmed death 1 ligand 1
pFOXO1	phosphorylated form of FOXO1
PI3K	phosphoinositide 3-kinase
PLCγ2	phospholipase C gamma 2
PMA	phorbol 12-myristate 13-acetate
pMHC	peptide-MHC complexes

PPAR-γ	peroxisome proliferator-activated receptor- γ
PTEN	phosphatase and tensin homolog
Rag	recombination activating gene
S1P1	sphingosine 1-phosphate receptor 1
SarAg	sarcoma antigen
TAMs	tumor-associated macrophages
TCF	T cell factor
T_{CM}	central memory T cells
TCR	T cell receptor
T_{EFF}	effector T cells
T_{EM}	effector memory T cell
TGF-β	transforming growth factor β
Th	T helper
TILs	tumor-infiltrating lymphocytes
TIM-3	T-cell Ig-and mucin-domain-containing molecule-3
TME	tumor microenvironment
TNF	tumor necrosis factor
T_{reg}	regulatory T
UTR	untranslated regions
VEGF	vascular endothelial growth factor
WNT	Wingless and INT-1

Fig. 1.

Roles of FOXOs playing in tumor and stromal cells that contribute to the immune response. The cell-intrinsic roles of FOXO1 in tumor cells, endothelial cells, as well as CAFs mainly shape anti-cancer immunity, extrinsically leading to immune activation. FOXOs inhibit the expression of PD-L1, CCL4, and VEGF-A through repressing Myc or Wnt/β-catenin signaling pathways in tumor cells. Furthermore, FOXOs restrain CAF proliferation but act as a gatekeeper to maintain CAF senescence. In addition, FOXO can inhibit the proliferation of endothelial cells and repress their capacity for angiogenesis. FOXO-mediated inhibition of PD-L1, CCL-4, and VEGF-A expression levels, as well as CAF cell quantity and angiogenesis can create a microenvironment that promotes immune responses against tumor cells. However, there are exceptions to this rule, as FOXO1 can also promote expression of VEGF-C in tumor cells.

CAFs: cancer-associated fibroblasts; PD-L1: programmed death 1 ligand 1; CCL4: chemokine (C-C motif) ligand 4; VEGF: vascular endothelial growth factor.

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FOXOs-mediated T cell Immunosuppression extrinsically via DCs and macrophages

Fig. 2.

Roles of FOXOs in regulating the function of dendritic cells (DCs) and macrophages. Upon activation by CCR7 ligand, Foxo1 acts as a DC suppressor, by inhibiting DC survival and promoting apoptosis. The level of Foxo3 activation that occurs during LCMV infection is sufficient to reduce levels of IL-6 and TNF- α . In addition, Foxo1 promotes the polarization of macrophages to M2-like phenotype when responding to LPS stimulation. The function of FOXOs in these processes in DCs and macrophages result in repression of T cell activation and expansion.

DCs: dendritic cells; CCR7: C-C chemokine receptor 7; LCMV: lymphocytic choriomeningitis virus; IL-6: interleukin-6; TNF- α : tumor necrosis factor- α .

Fig. 3.

FOXOs regulate anti-cancer immunity via mediating crosstalk between tumor cells and immune cells. In immune tissue of healthy individuals, FOXOs promote B cell differentiation, as well as the generation of both naïve and inducible T_{reg} cells, M2 type macrophages, and memory $CD8^+$ T cells. In contrast, FOXOs negatively regulate B cell proliferation as well as NK cell maturation and function, while simultaneously repressing the generation of T_H1 cells, T_H2 cells, effector $CD8^+$ T cells, M1 macrophages. In intratumoral stroma during cancer development, the loss of FOXO activity in tumor cells and endothelial cells contributes to the establishment of immunorepressive

microenvironments in tumor stroma, via promoting secretion of several immunosuppressive factors, such as VEGF and PD-L1. Furthermore, constitutive reduction of Foxo1 activity in T_{reg} cells leads to accumulation of activated T_{reg} cells in tumor stroma, which acts as a strong repressor of CTL responses. However, in CD8⁺ T cells and NK cells, the activity of FOXO transcription factors becomes down-regulated upon encounter of tumor cells, thus leading to enhanced anti-tumor activity.

M1: Type 1 macrophage; M2: Type 2 macrophage; T_H1: Type 1 helper cells; T_H2: Type 2 helper cells; T_{reg}: regulatory T cells; DC: dendritic cell; VEGF: vascular endothelial growth factor; CCL4: chemokine (C-C motif) ligand 4. (–): Inhibition; (+): Activation.

Table 1

FOXOs targeted genes in the setting of cancer immunity

Cell Type	Function	FOXO-Regulated Genes	References
Tumor cells	Immunosuppressive factor	PD-L1 ↓; CCL4 ↑	[17, 18] [27]
Tumor cells	Angiogenesis	VEGF ↓ (FOXO3)	[22]
Endothelial cells	Angiogenesis	eNOS ↓, ELK-3 ↓ (FOXO1 and 3); MYC ↓ (FOXO1); p27 ↑ (FOXO3); CDK2, cyclin D1 and PCNA ↓ (FOXO3); BMP4 CXCR4, stabilin-1, PDGF-β ↑ (FOXO1 and 3); THBS1 ↑ (Foxo1, 3, 4)	[32, 33, 35, 40]
Fibroblasts	Proliferation and senescence	PDGF ↓ (Foxo1 and 3)	[46]
DCs	Homing, Survival and activation	CCR-7, ICAM-1, Bim ↑ (Foxo1); IL-6 ↓ (Foxo3)	[52–54, 56, 57]
Macrophages	Homeostasis of M1 and M2 differentiation	IL-10, Arg1, Fizzl, IL-13ra1 ↑; iNOS, IL-12a, IL-12b, CCR2 ↓ (Foxo1)	[61]
T cells	Naïve T cell homing, trafficking and survival	S1P1, CCR7, CD62L, CD127 ↑ (Foxo1); p27Kip1; Bcl-2 ↑ (Foxo3)	[62–66]
	T _H 1/2 cell differentiation	IL-2, IFN-γ ↓; IL-4, IL-5, IL-6, IL-10 ↑ (Foxo1 and 3)	[65, 67]
	Naïve and inducible T _{reg} cell generation and function	Foxp3, CD25, CTLA-4 ↑; CD62L ↑ (Foxo1,3)	[14, 73, 74, 76, 77]
	CD8 ⁺ T cell effector and memory	T-bet ↓ Eomes, CD62L, CCR7, PD-1 ↑, BCL2 ↑ (Foxo1)	[96–99, 101, 102]
NK cells	Maturation and function	T-bet ↓, CD62L ↑ (Foxo1)	[118]
B cells	Proliferation and differentiation	cyclin G2, p130, KLF4, CD127, Rag1, Rag2, CD62L ↑ (Foxo1,3);	[123–128]