Forkhead Box Transcription Factors: Double-Edged Swords in Cancer

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ABSTRACT

A plethora of treatment options exist for cancer therapeutics, but many are limited by side effects and either intrinsic or acquired resistance. The need for more effective targeted cancer treatment has led to the focus on forkhead box (FOX) transcription factors as possible drug targets. Forkhead factors such as FOXA1 and FOXM1 are involved in hormone regulation, immune system modulation, and disease progression through their regulation of the epithelial–mesenchymal transition. Forkhead factors can influence cancer development, progression, metastasis, and drug resistance. In this review, we discuss the various roles of forkhead factors in biological processes that support cancer as well as their function as pioneering factors and their potential as targetable transcription factors in the fight against cancer.

Introduction

Current options for cancer treatment include surgery, radiotherapy, chemotherapy, and more recently developed targeted immunotherapies and checkpoint blockade therapies (1, 2). Both radiation and chemotherapy have a plethora of limitations, including side effects that require that clinical benefits be weighed against toxicity and the emergence of resistance that may result from intratumor heterogeneity (1–3). Targeted treatments also often cause side effects, including but not limited to gastrointestinal symptoms, and cancer frequently recurs or progresses despite treatment (3). Cancer metastasis and recurrence account for almost all cancer-associated deaths (4). Patients with advanced-stage cancer have a poor prognosis and high rates of resistance to available treatments (4).

There has been growing interest in the forkhead box (FOX) family of transcription factors as targets for anticancer drug development. Members of this family have an evolutionarily conserved DNA-binding domain and are involved in the regulation of cell growth, differentiation, and embryogenesis (5). In various cancers, including ovarian, breast, and prostate cancer, FOX factors are implicated in cancer initiation, progression, and chemoresistance (6–9). In this review, we discuss the roles of FOX factors in the epithelial–mesenchymal transition (EMT), hormone signaling, drug resistance, metabolism, and immune system regulation and their functions as pioneering factors. Based on our current understanding of the intertwined and complex roles of FOX proteins in cancer development and progression, these factors are important emerging therapeutic targets.

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EMT, EM Plasticity, and Cancer Stem Cell Properties

EMT is at the epicenter of cancer progression (10). The transition from the epithelial phenotype to the mesenchymal-like phenotype is a dynamic process necessary during normal development that is also involved in tumor progression and the acquisition of resistance to chemotherapy (10). EMT involves the reorganization of the epithelial cell cytoskeleton, loss of cell–cell junctions and apical–basal polarity, and a change in signaling programs that alter the expression of proteins defining cell shape (**Fig. 1A**) (11). In addition, EMT endows cancer cells with stem cell–like properties (12). Cancer stem cells (CSC) are a rare subtype of cancer cells that are intrinsically resistant to chemotherapy that are implicated in disease recurrence. Therapeutic agents that target transcription factors, such as the FOX proteins that regulate EMT, may enable the inhibition of metastasis, eradication of CSCs, and reversal of drug resistance.

FOXC2 is the central mediator of EMT through the activation of various signaling pathways, including MAPK and PI3K/AKT, or by modulation of other transcription factors such as FOXO3 (13–15). We have also shown that FOXC2 results in the enrichment of CSC populations, thereby linking EMT to the induction of CSCs (12, 16). This leads to a chemoresistant state in various cancer types (6, 17, 18). The interconnectedness of CSCs, EMT, and drug resistance through FOXC2 places this transcription factor at the center of cancer progression and recurrence. Thus, the specific inhibition of FOXC2 is an attractive treatment option. Several methods of FOXC2 downregulation have been studied. For example, p38 inhibition, which leads to a decrease in FOXC2 levels, blocks the migratory and invasive capabilities of cancer cells (16).

Like FOXC2, FOXC1 has been linked to the induction of EMT (19, 20). FOXC1-mediated activation of the EMT program occurs through the transactivation of the transcription factor Snail and direct activation of ZEB2, both well-known EMT regulators (21, 22). FOXC1 is also able to activate the PI3K/AKT pathway (22), which, in a mechanism similar to that of FOXC2, leads to the downregulation of FOXO3 and the emergence of a chemoresistant state.

Several other FOX factors also influence EMT progression. The FOXA proteins are members of the class of transcription factors known as pioneering factors; these are transcription factors that associate with compacted chromatin to mediate binding of other transcription factors. The loss of FOXA family members affects the
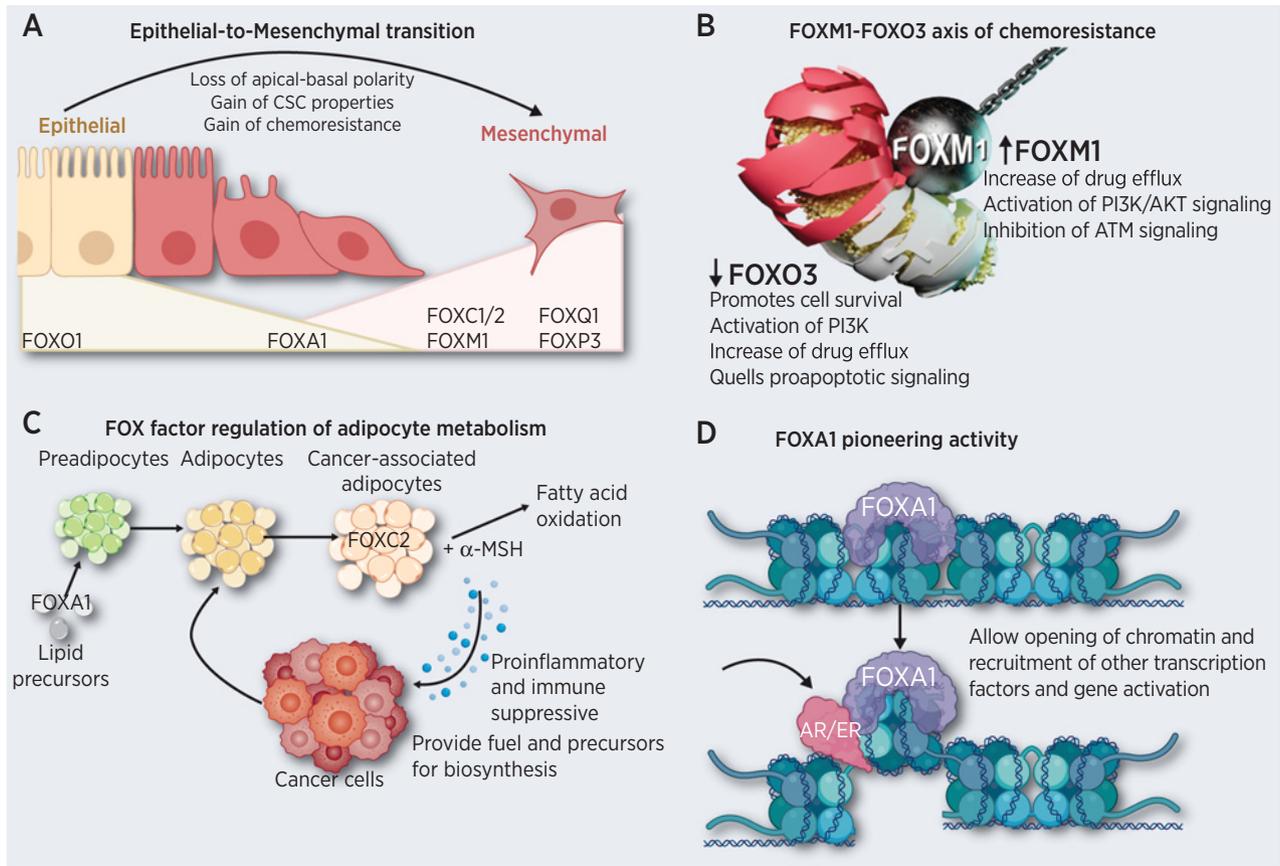
Figure 1.
Role of FOX factors in cancer. **A,** Depiction of the two extreme phenotypes of EMT with the associated physiologic changes and the FOX factors that elicit each phenotype. FOXA1 can induce and inhibit EMT, depending on the cancer subtype. **B,** Summary of the functions of the FOXO3–FOXM1 axis in drug resistance. A drug-resistant state occurs upon inhibition of FOXO3 or upregulation of FOXM1, often in combination, leading to an increase in prosurvival mechanisms and drug efflux transporter activation. **C,** Schematic of how FOX factors regulate adipocyte metabolism, creating an immunosuppressive environment. FOXA1 mediates the acquisition of lipid precursors to fuel tumor proliferation. These precursors can become cancer-associated adipocytes expressing FOXC2. The cooperation between FOXC2 and α-MSH to promote fatty-acid oxidation creates an energy source for cancer cells. This creates a feedback loop in which cancer cells signal adipocytes to become cancer-associated adipocytes, and the cycle continues. **D,** Pioneering activity of FOXA1, a potential mechanism of action of other transcription factors. FOXA1 is capable of binding condensed chromatin to create an opening for easier accessibility of other transcription factors. In this case, FOXA1 allows for the binding of ER or AR to condensed chromatin, thereby activating an altered hormone response in cancer cells.

accessibility of enhancer regions of epithelial genes, triggering a switch from epithelial to mesenchymal gene expression (23, 24). The precise effect of FOXA factors on EMT induction is tissue-specific: In prostate cancer, the inhibition of FOXA leads to EMT inhibition, whereas loss of FOXA1 is necessary for EMT induction in pancreatic and breast cancer (25–27). The link between FOX factors and EMT is multifaceted. The varying induction of EMT through FOXA1 can be partially attributed to the reversibility element associated with EMT and to the intertwined programs such as EMT and metabolism and hormone regulation. FOXA1 is also involved in the regulation of androgen receptor (AR) and estrogen receptor (ER) signaling. This complex system of regulation by FOXA1 is potentially the cause of its dual roles in EMT induction.

Two FOX factors, FOXM1 and FOXO, are closely linked to drug resistance and are also mediators of EMT. Depletion of FOXM1 inhibits the proliferative and invasive capabilities of cancer cells, and its overexpression is linked to EMT induction (28–30). Several FOXM1 binding sites have been identified in the promoter of *Slug*, a well-characterized EMT-inducing transcription factor (31). Unlike

FOXM1, which induces EMT and leads to a chemoresistant state, FOXO1 inhibits EMT. FOXO1 inhibits TGFβ–induced EMT, negatively regulates EMT transcription factors, and interacts with ZEB2 (32). In pancreatic ductal carcinoma, FOXO depletion induces EMT via activation of the β-catenin/transcription factor 4 (TCF4) pathway, a known CSC-associated signaling pathway (33).

Other FOX factors are also linked to EMT induction. FOXP3 induces EMT and promotes tumor growth by activating β-catenin/Wnt-mediated signaling by binding directly to β-catenin and by activating NF-κβ signaling, a regulatory pathway involved in inflammatory responses and cell survival (34–36). FOXQ1 induces signaling mediated by TGFβ to initiate EMT (37, 38) and represses the expression of E-cadherin and induces mesenchymal properties through induction of ZEB2 (37, 39). FOXF2 induces EMT induction and cancer progression with induction of bone metastasis seen in breast cancer (40, 41). However, FOXF2 induction is cancer-specific (42). Thus, various FOX factors are involved in EMT induction (**Fig. 1A**) and acquisition of CSC traits and the mesenchymal phenotype. These examples also demonstrate that the actions of FOX factors in cancer

initiation and progression are complex. The prevalence of FOX factors in the induction of EMT sets the stage for their involvement in various cancer-associated pathways due to the complex role of EMT in cancer progression. It remains to be seen whether targeting these transcription factors alone will be enough to halt or reverse EMT or whether this process is so complex that a multifaceted approach is necessary.

Regulation of Hormone Signaling

In order to understand how FOX factors influence cancer progression, we must examine their roles in the regulation of hormone signaling pathways. Hormone-receptive cancers are often treated with therapies that target the relevant hormone signaling pathway. FOX factors are intricately involved in ER, HER2, and AR signaling. Hormone signaling is abnormal in over 60% of breast cancers (43). Targeting of hormone-mediated signaling pathways can be an effective treatment option; however, endocrine treatment (e.g., tamoxifen or aromatase inhibitors) often leads to treatment resistance and recurrence (44).

A key determinant of the response to estrogen is the level of FOXA1 expression (45). FOXA1 can activate ER-mediated signaling (46), which is associated with the activation of various protein kinase cascades and signaling pathways involved in cell proliferation and survival (47). In addition, this genome-wide reprogramming drives the activation of the hypoxia-inducible factor-2α (HIF-2α) transcription factor and its induction of a prometastatic program in breast cancer (23).

FOXM1 and FOXP1 also function in endocrine sensitivity and resistance in breast cancer. FOXM1 is correlated with ER and HER2 expression, and FOXM1 is able to activate ER expression by binding to forkhead-response elements located in the proximal region of the *ER* promoter (48–50). In addition, FOXP1 increases recruitment of ERα to ER binding sites to increase cellular proliferation (51, 52). FOXM1 can bypass the need for ER in downstream ER signaling pathways, resulting in endocrine resistance and tumor growth in breast cancer as well as an increase in CSC frequency (7, 53, 54). In addition, FOXM1 expression is dependent on HER2 expression, making it a likely downstream target of HER2 signaling (55). The functions of FOXM1 in both ER and HER2 signaling allow for a switch away from hormone-dependent signaling and make FOXM1 a prime target to combat acquired endocrine resistance in patients with cancer.

FOX factors can also regulate AR signaling. Prostate cancer cell proliferation is often dependent on AR-mediated signaling, and androgen deprivation therapy (ADT) is the first line of therapy for these tumors (56, 57). AR is required for normal male physiology and is also expressed in prostate cancer cells. ADT often fails in advanced stages of prostate cancer, which is termed castration-resistant prostate cancer (CRPC) (25, 58). Members of the FOX family of transcription factors are critical in AR-mediated signaling, particularly in CRPC (59).

In addition to regulating ER-mediated signaling, FOXA1 mediates AR expression (60). The forkhead domain of FOXA1 binds to sequences on the *AR* gene and physically interacts with AR, acting as a pioneering factor to recruit the AR protein. Thus, FOXA1 positively regulates prostate-related gene expression induced by androgens. It is expressed at high levels in patients with metastatic prostate cancer (60). Much like FOXA1, FOXA2 interacts with AR, resulting in activation of the promoter of the gene encoding the prostate-specific antigen (PSA). PSA, in turn, regulates the growth of epididymal cells, which are required for prostate physiology (61). Both FOXA1 and FOXA2 can upregulate AR-targeted genes in the absence of AR (62, 63), increasing prostate cancer growth, even in the absence of androgens, leading to the failure of ADT.

Other FOX transcription factors can also regulate AR-mediated signaling. For example, FOXM1 influences the binding of AR to the *CDC6* promoter, increases the AR protein levels and induces PSA gene transcription in the absence of androgen stimulation (64, 65). FOXO3 can directly bind to AR promoter and the function of FOXO3 is regulated by the PIK3/AKT pathway (66). In contrast to these positive regulators of AR-mediated gene expression, FOXP1 is able to repress AR target genes (67). FOXP1 functions as a homodimer or heterodimer with FOXP2 and FOXP4 (67). When AR enters the nucleus, it is ligand-dependently recruited to bind FOXP1, leading to the negative regulation of AR target genes (67). In summary, FOX factors are essential players in hormone signaling in various cancer types with roles as both tumor promoters and suppressors. The involvement of FOX factors in hormone signaling links hormone regulation to drug response in cancer. Most importantly, FOX factors create hormone-independent states in which cancer cells can bypass the typical hormone regulatory pathways needed for normal physiologic growth, such as those seen in breast and prostate cancer. This scenario allows for unsuppressed growth and cancer progression.

Resistance to Therapeutics

Drug resistance remains a major hurdle in cancer treatment (2, 3). Drug resistance can develop through acquired resistance to drug regimens, tumor heterogeneity, or altered immune system intervention (68). In hormone-receptive cancers, single-use agents to target hormone signaling have been shown to be ineffective long term because of acquired resistance, as these agents cause a selective pressure that increases the frequency of CSCs (68). The drug resistance mechanisms include the development of resistance to apoptosis, increased DNA damage repair efficiency, elimination of the chemotherapeutic drugs by increased efflux, and drug inactivation.

Several of the FOX factors involved in the regulation of hormone signaling and EMT, including FOXO3 and FOXM1, are also implicated in drug resistance. FOXO3 depletion induces the expression of proteins important for drug efflux (such as MDR1) and proliferation in cancer, mostly through the activation of the PI3K signaling pathway, a well-known cancer-driving signaling pathway (69, 70). The inactivation of FOXO3 is due to the hyperactivation of the PI3K/AKT pathway, which results in the cytoplasmic translocation and degradation of FOXO protein (71). The degradation of FOXO proteins leads to a reversal of the proapoptotic properties of FOXO family members (13, 72, 73).

Although inactivation of FOXO3 alone can induce drug resistance, the FOXO3–FOXM1 axis is a better marker of drug resistance modulation (**Fig. 1B**; refs. 74, 75). FOXM1 and FOXO3 compete for the same binding sites in gene promoters, and FOXO3 can negatively regulate FOXM1 expression (76). When FOXM1 is upregulated, or FOXO3 is downregulated, the expression of genes involved in drug efflux, DNA repair, and cell survival are impacted (75). FOXM1 transcription is activated by an increase in reactive oxygen species (ROS), creating a feedback loop between ROS and FOXM1 expression (77). This leads to regulation of cell-cycle transition points by FOXM1 and increased resistance via overexpression of DNA damage response genes and the development of resistance to DNA damaging agents (78, 79). This acquired resistance may be due to the expansion of

CSCs induced by FOXM1 (80–82). By acting synergistically with FOXO3 inactivation, FOXM1 blocks the action of trastuzumab by increasing the proteolytic degradation of p27, an inhibitor of cell-cycle progression (7, 78). FOXM1 creates a perfect axis for drug resistance by increasing the number of resistant CSCs, effectively pumping out drugs from the cell, and downregulating the expression of genes that serve as tumor suppressors.

Recently, another member of the FOX family, FOXC2, has been shown to be important in drug resistance and metastasis in multiple cancer types, including those of the prostate, breast, and ovary (83–85). High levels of FOXC2 expression are observed in advanced metastatic cancers, and its expression is correlated with poor prognosis (86). Inhibition of FOXC2 expression by treating cells with an inhibitor of p38 kinase, an upstream regulator of FOXC2, reduces resistance to chemotherapy agents (85). In accordance with these data, in ovarian cancer, the inhibition of FOXC2 expression increases chemosensitivity by decreasing the concentrations of phosphorylated forms of AKT and ERK protein kinases, which are involved in multiple cellular processes tied to cell proliferation and migration (6). In summary, FOXC2 is able to upregulate not only cell proliferation and migration but also chemoresistance through its modulation of EMT.

FOXQ1 is also involved in drug resistance. Elevated FOXQ1 expression is linked to poor prognosis in patients with cancer patients (87). FOXQ1 upregulation leads to the activation of PDGFR-α and PDGFR-β, upregulation of Twist and ZEB2, and initiation of EMT (88, 89). All of these proteins are involved in cancer progression and the generation of a drug-resistant state. FOXQ1 has been shown to control the expression of MDM2, which is a negative regulator of the expression of the tumor suppressor p53 (87). Thus, FOXQ1 upregulates EMT-involved transcription factors and downregulates tumor suppressor expression, leading to drug resistance. In summary, a large body of evidence points to multiple mechanisms by which various FOX transcription factors can confer chemoresistant traits to cancer cells, with factors involved in hormone signaling and EMT induction being closely tied to the induction of chemoresistance.

Cancer Metabolism

In addition to chemoresistance, FOX factors influence cancer growth through metabolic regulation. Cancer cells depend on aerobic glycolysis, a process known as the Warburg effect (90, 91). Normal cells utilize oxidative phosphorylation; however, in tumors, a hypoxic environment creates an environment in which oxidative phosphorylation is inefficient. Thus, cancer cells resort to glycolysis, in which two ATP molecules and intermediate building blocks are produced for every glucose consumed (92). Even though the production of ATP from glycolysis is lower on a molar basis than that of oxidative phosphorylation under normal conditions, the amount of ATP produced by a cancer cell can equal or even surpass that produced by a cell through oxidative phosphorylation (91, 93). Through glycolysis, cancer cells can generate an adequate amount of energy while also creating intermediates needed to promote cell growth. Two FOX factors have been implicated in the regulation of the Warburg effect: FOXM1 and FOXO.

FOXM1 has been shown to promote the Warburg effect and regulate glycolysis by upregulation of the expression of the lactate dehydrogenase (LDHA; refs. 94, 95). The resulting elevation of lactate production and glucose consumption causes an increase in glycolytic flux, fueling cancer cell growth. In addition, FOXM1 has been shown to upregulate the expression of the glucose transporter GLUT1 and the kinase HK2, which catalyzes the first step in glucose metabolism, further stimulating the Warburg effect (95). In contrast, FOXO has been shown to inhibit glycolysis through its antagonization of MYC (96). MYC is an oncogene that promotes a shift in metabolism primarily to glycolysis. Elevated FOXO levels lead to a decrease in ROS and a shift away from glycolysis (96). Thus, the FOXM1–FOXO axis, which plays a role in drug resistance, also plays a critical role in the metabolic shift in cancer cells.

Aerobic glycolysis leads to the production of the precursors needed for the biosynthesis of lipids and adipocytes; both have been shown to promote cancer metastasis (97, 98). Adipocytes store lipids and also secrete proinflammatory and immune-suppressing molecules. When adipocytes are close to cancer cells, they are reprogrammed to secrete proinvasive and prometastatic signals (98). The cancer-associated adipocytes can also provide cancer cells with energy sources through lipolysis (98). Thus, lipids and adipocytes create an environment that induces survival and metastasis.

FOX factors mediate adipocyte function (**Fig. 1C**). FOXA is expressed in preadipocytes and regulates the expression of LIPG, an enzyme responsible for generating lipid precursors (99, 100). Thus, FOXA mediates the acquisition of extracellular lipids that fuel tumor proliferation (99). FOXC2 is involved in lipid and adipocyte metabolism (101) and cooperates with α-MSH to promote fatty acid oxidation, creating an energy source for cancer cells (102). FOXC2 suppresses apoptosis in preadipocytes and promotes adipogenesis, another mechanism of synthesis of precursors that cancer cells utilize for energy and biosynthesis (103). In addition, FOXC2 promotes glycolysis by regulating the YAP/TAZ pathway, leading to an increase in tumor progression (104). Thus, FOX factors regulate cancer cell metabolism and adipogenesis, providing cancer cells with energy sources and creating a proinflammatory, prosurvival, and prometastatic environment. The interplay between FOX factors that regulate EMT, chemoresistance, and cancer metabolism demonstrates the complex and interconnected nature of signaling pathways involved in cancer progression. Based on this, a valid concern is that inhibiting the function of one of these FOX transcription factors, such as targeting FOXC2-regulated metabolism, will not be sufficient to halt cancer progression.

Evasion and Regulation of the Immune System

In addition to a prosurvival and metastatic environment, FOX factors can regulate the immune system to allow cancer cells to evade immune detection. Some FOX factors inhibit the immune response and others activate senescence. Senescence, the arrest of the proliferation of cells, leads to a change in the microenvironment that can allow tumor development (105). Senescent cells secrete numerous proinflammatory cytokines, chemokines, growth factors, and proteases that allow cancer cells to evade the immune system (105). Recovery from a senescent state after exposure to therapy is called proliferative recovery, and it has been shown to contribute to cancer recurrence (106). Senescent cells are not targetable with existing therapies because they do not actively proliferate (106).

Several FOX factors contribute to the induction of senescence. FOXM1 is a key regulator of senescence induction through regulation of the G_2–M transition (107). Depletion of FOXM1 leads to the induction of a senescent state in multiple cancer cell types (107–109). FOXM1 downregulation also causes an increase in the abundance of ROS and the downregulation of c-MYC in cancer cells (107, 110). This,

in turn, limits mitosis and increases the release of prosenescence markers that ultimately halt proliferation (108).

FOXO4 also has a demonstrated function in senescence. The activation of FOXO4 is correlated with an increase in levels of p21 protein and an increase in the population of senescent cancer cells (111, 112). FOXO4 binds to p53 at regions of DNA damage, enabling p53 to bind and transactivate the *p21* promoter (111, 113). p21 executes the senescence program by causing cell cycle arrest (114). The arrest in the cell cycle leads to a survival advantage in the presence of chemotherapeutic drugs. A better understanding of how FOX factors initiate senescence through the upregulation of an inflammatory environment and inhibition of cell cycle regulators will guide the development of targeting strategies to overcome this phenomenon.

Although FOXQ1 is not as well characterized as some other FOX factors, it is known to induce EMT and has recently been shown to play a role in the repression of cancer senescence (115). FOXQ1 is responsible for the induction of proliferation through its suppression of IL6 and IL8 through the modulation of sirtulin 1 (115). IL6 and IL8 are proinflammatory chemokines responsible for senescence induction, and the inflammatory environment created by IL8 and IL6 further enhances survival. Downregulation of FOXQ1 induces premature senescence in fibroblasts, and its overexpression thwarts senescence induction (115). Thus, additional studies should be performed on FOXQ1 to determine its relationship to immune system modulation, as FOXQ1 may inhibit cancer growth by reducing inflammation.

Immunomodulatory cancer therapies have been developed that augment patients' immune responses to cancer cells. Several FOX factors modulate key immune system signaling pathways, leading to an interest in their use as immunotherapy agents. Regulatory T (Treg) cells are an immunosuppressive subset of $CD4^+$ T cells that account for about 5% of the $CD4^+$ T-cell population (116). Treg cells are prevalent in tumor samples, and they are early responders during tumor development that are associated with poor patient prognosis (116, 117). Th cells are the primary line of adaptive immunity, activating B cells and cytotoxic T cells (118). Thus, helper T cells have functions opposite those of Treg cells in cancer progression.

FOXO3 can inhibit inflammatory transcriptional activity by blocking NF-$\kappa\beta$ activation, leading to inhibition of T_h-cell function and immune tolerance (119). FOXO3 expression is increased after T-cell receptor engagement and is involved in T_h-cell differentiation (120). FOXO3 positively affects immune system activation, whereas FOXO3 inhibition has been linked to drug resistance acquisition. FOXO1 induces the production of T_{reg} cells by binding to the FOXP3 promoter and triggering robust FOXP3 expression (121). FOXP3 is considered to be the master regulator of Treg cells (122). Sustained FOXP3 expression is required for the proper function of Treg cells (123). In addition, FOXP3 induces an immunosuppressive environment by repressing cytokines such as IL4 that promote a proper immune response (124, 125). Thus, FOXO1 and FOXP3 work together to elicit an immunosuppressive, prosurvival microenvironment, leading to the activation of Treg cells. The emergence of these immunosuppressive environments leads to the inhibition or resistance to immune checkpoint blockade therapy. Targeting the FOX family of transcription factors in combination with standard-of-care chemotherapeutics can aid in combating chemoresistance, one of the biggest hurdles of cancer treatment. Thus, a double targeted approach of chemotherapeutics plus FOX factor-targeting compounds could inhibit the bulk of the tumor, target the CSC population, and inhibit the emergence of resistance.

Pioneering Factors

A fundamental reason for targeting FOX proteins is that many FOX proteins function as pioneering factors. Pioneering factors are the first to engage at target sites on chromatin and therefore serve to initiate transcriptional programs (126). Under normal conditions, most transcription factors do not directly access their target sites on compacted chromatin but rather bind after the binding of a pioneering factor (126, 127). Pioneering factors are crucial for cellular reprogramming and are also implicated in gene regulatory networks that occur during cancer progression (128). Under some conditions, pioneering factors can activate regulatory programs that result in cancer cell propagation without normal checks and balances that restrict proliferation. The FOX proteins have a highly conserved winged-helix DNA-binding domain that resembles a linker histone and allows binding to nucleosomes (129).

FOXA1 and FOXA2 are capable of displacing histone H1, and they maintain enhancer regions in an open conformation, allowing other transcription factors to bind and activate gene expression (128, 130). The FOXA factors can activate redundant pathways despite the loss of any individual transcription factors (131). Loss of hormone production in hormone-dependent cancers, which should halt cancer progression, is thwarted as FOXA factors turn on otherwise hormone-dependent pathways in the absence of hormone secretion. For example, FOXA1 has been shown to open compact chromatin to facilitate AR recruitment to maintain the prostate epithelial phenotype and regulate the expression of genes encoding AR and ER (**Fig. 1D**; refs. 62, 132). In addition, FOXA proteins remain bound to chromatin during mitosis and help to activate signaling pathways that regulate cell fate (**Fig. 1D**; ref. 133). More studies are needed to explore the roles of FOXA transcription factors in cell fate determination during cancer progression.

Another FOX factor with pioneering capabilities is FOXO1, which opens and remodels chromatin via its conserved winged-helix domain (134). FOXO1 and FOXA1/2 cooperate to open condensed chromatin around insulin-regulated genes (135). FOXO1 decondenses chromatin arrays to regulate metabolism, cell survival, apoptosis, cell-cycle progression, and DNA repair (136). FOXI1 associates with mitotic chromatin and remains bound throughout mitosis (137, 138) but little is known about the role of this factor in cancer progression. FOXM1 expression has been shown to be required for proper chromosome segregation and mitotic progression (139, 140).

FOXC2 regulates mitotic entry via PLK1, a kinase responsible for G_2–M regulation, and levels of FOXC2 fluctuate during cell-cycle progression, a known characteristic of mitotic bookmarkers (141). Mitotic bookmarkers are a subset of pioneering factors that possess the ability to bind to chromosomes during mitosis to rapidly activate a subset of genes following mitotic exit. Additional studies are needed to determine whether other FOX factors are both pioneering factors and mitotic bookmarkers. The ability of FOX factors to impact a plethora of critical pathways and regulatory mechanisms involved in cancer progression is likely due to their roles as pioneering factors that regulate cell-fate determination and acquisition of CSC properties. FOX factors are not only involved in the regulation of cancer cell signaling but also in the activation of these pathways through the regulation of chromosome accessibility. Thus, FOX factors may control more than just the cancer signaling pathways; they may also be involved in the epigenetics and cell fate control during cancer progression.

Table 1. Functions of FOX factors in cancer.

FOX factors	Roles in cancer
FOXA1 and FOXA2	Bind to sequences on AR gene (58, 61)
	Open compact chromatin for AR and ER recruitment (45, 58, 132)
	Duality in EMT activation, cancer (26, 58)
	Promote lipid precursor accumulation (99)
	Pioneering factors (130)
FOXM1	Increases AR levels via recruitment of AR to CDC6 in the absence of androgen stimulation (65)
	Correlates with ER and HER2 expression (48, 65, 80)
	Binds to forkhead response elements located in the proximal region of ER promoter (49)
	Increases drug resistance via overexpression of DNA damage response genes (70, 75, 76)
	Initiates EMT through binding of Slug promoter (31)
	Induces senescence via regulation of cell division at G_2–M (107, 109)
	Promotes Warburg effects (94)
FOXO3	Binds to AR promoter (66)
	Regulates cell fate via apoptosis induction (13, 73)
	Upregulates drug efflux and activation of the PI3K pathway (13, 14, 72, 75)
FOXC2	Upregulates EMT, CSC population, and AKT signaling, leading to drug resistance (16, 80, 97)
	Enriches the CSC population in tumors (16)
	Mediates G_2–M transition (141)
	Promotes fatty-acid oxidation (102)
FOXP1	Represses AR-targeting genes via the formation of dimers with FOXP2 and FOXP4 (67)
FOXO4	Increases p21, a senescence marker (113)
	Binds to p53 at the area of DNA damage and activates the p21 promoter (113)
FOXO1	Stimulates dendritic cells and T and B lymphocytes (121)
	Binds to FOXP3 promoter and regulates the production of Treg cells (135)
	Elicits an immunosuppressive environment (121)
	Pioneering factor (136)
FOXP3	Binds directly to β-catenin and upregulates GSK3β (34)
	Maintains proper function of Treg cells (122, 123, 125)
FOXQ1	Activates PDGFR-α and β, Twist, and ZEB2, leading to EMT induction (37, 38, 88)
	Controls expression of MDM2, a regulator of p53 (87, 89)
	Induces proliferation through suppression of IL6 and IL8 (115)
FOXF2	Promotes bone metastasis in breast cancer (40)
	Induces EMT and cancer progression, cancer-specific (41, 42)

Future Directions

Our ability to successfully treat cancer has improved over the last decade; yet, there is no cure for highly invasive or metastatic cancers. Studies of FOX factors have provided unique perspectives into the mechanisms that drive cancer progression. Often, FOX factors are involved in more than one aspect of cancer progression (**Table 1**). Clarification of the functions of FOX factors in cancer modulation and the identification of druggable targets that alter the activities of these transcription factors could lead to additional options for the treatment of cancer. Inhibition of FOX factors, or the pathways they mediate, can potentially reverse drug resistance, inhibit immune evasion, and block metastatic progression. Inhibitors of FOX factors, the kinases involved in FOX-mediated pathways, and the agents that mediate RNAi to silence the expression of FOX factors are promising tools to treat patients with various types of cancer.

However, additional studies are required to shed light on the complexity of FOX factors. The dual natures of certain FOX factors during EMT raise the concern that inhibition of these transcription factors during different stages of cancer progression could lead to undesirable outcomes (42, 142). In addition, certain FOX factors have contradictory outcomes depending on the tissue. For example, FOXA has inhibitory effects in pancreatic cancer but stimulatory effects in breast and prostate cancers (25, 26). This duality in EMT and metastatic progression may be due to the intricate nature of FOXA's involvement with hormone signaling.

Of particular interest will be the study of the abilities of FOX factors to function as pioneering factors. Their highly conserved winged-helix DNA-binding domain mimics a histone linker, and therefore, it is well-matched to the binding of condensed chromatin structures (129). All FOX factors may have the pioneering ability, although this remains to be experimentally confirmed. Their abilities to serve as pioneering factors allow FOX factors to activate compensatory mechanisms needed for cancer growth as well as to determine cancer cell fate. FOX transcription factors are linked to EMT, hormone signaling, drug resistance, metabolic reprogramming, and immune system modulation, and their study may be key to understanding cancer.

Authors' Disclosures

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