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FOXC1 in cancer development and therapy: deciphering its emerging and divergent roles

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***Abstract*:** Forkhead box C1 (FOXC1) is an essential member of the forkhead box transcription factors and has been highlighted as an important transcriptional regulator of crucial proteins associated with a wide variety of carcinomas. FOXC1 regulates tumor-associated genes and is regulated by multiple pathways that control its mRNA expression and protein activity. Aberrant FOXC1 expression is involved in diverse tumorigenic processes, such as abnormal cell proliferation, cancer stem cell maintenance, cancer migration, and angiogenesis. Herein, we review the correlation between the expression of FOXC1 and tumor behaviors. We also summarize the mechanisms of the regulation of FOXC1 expression and activity in physiological and pathological conditions. In particular, we focus on the pathological processes of cancer targeted by FOXC1 and discuss whether FOXC1 is good or detrimental during tumor progression. Moreover, FOXC1 is highlighted as a clinical biomarker for diagnosis or prognosis in various human cancers. The information reviewed here should assist in experimental designs and emphasize the potential of FOXC1 as a therapeutic target for cancer.

Keywords: biomarker, cancer metastasis, cell proliferation, forkhead box C1, tumor

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Introduction

Transcription factors have been implicated in controlling extensive gene expression and regulating various cellular responses.[1] Within the past two decades, understanding of the functions of transcription factors during tumorigenesis, which can alter the expression programs of multiple oncogenes or tumor suppressor genes, has increased greatly. Although transcription factors are promising targets for clinical therapies, they remain largely unexplored.[2] Forkhead box transcription factors (FOXs) are a family of evolutionarily conserved transcriptional factors characterized by a common DNA-binding domain (DBD) termed the forkhead box or winged helix domain.[3] To date, approximately 50 *FOX* genes have been identified in the human genome and 44 have been identified in mice, all of which can be further categorized into 19 subgroups (*FOXA* to *FOXS*).[4,5] Indeed, FOXs are critical for a wide variety of developmental and homeostatic processes of tumors, including proliferation, differentiation, apoptosis, metastasis, and invasion.[5] Given that FOXs control these multiple important processes, it is not unexpected that deregulation and mutation of FOXs can have an influence on cellular fate and even result in tumorigenesis. Although our knowledge of FOXs is still in its infancy, certain FOX subfamilies such as FOXA, FOXO, FOXM, and FOXP have been reportedly implicated in the genesis and progression of various cancer and serve as potential therapeutic targets for these cancers.[6]

Notably, members of class C, including FOXC1 and FOXC2, appear to play novel roles in cancer progression.[7–9] Nevertheless, many aspects of FOXC1 function remain undefined or not fully understood. Forkhead box C1 (FOXC1), located at chromosome 6p25, was one of the first human forkhead genes to be studied.[10,11] FOXC1 was

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initially demonstrated as a crucial transcription factor that regulates the development of embryos derived from the neural crest. Mutations of *FOXC1* have been generally accepted as a primary cause of Axenfeld–Rieger syndrome.[12–14] In addition to its role in embryonic development, FOXC1 has recently emerged as a possible primary regulator in a wide spectrum of human cancer, such as basal-like breast cancer (BLBC),[8,15,16] hepatocellular carcinoma (HCC),[17] non-small cell lung cancer (NSCLC),[18] ovarian cancer,[19] and nasopharyngeal carcinoma (NPC)[20] (Table 1). Clinical studies have shown that upregulation of FOXC1 may intensify cancer cell invasion and indicate a poor prognosis in patients.[8,15–18,21] In this review, we discuss the molecular mechanisms regulating FOXC1 and highlight current understanding of its role as a crucial transcription factor in cancer development, its potential as a biomarker for diagnosis and prognosis, and its possible therapeutic modalities.

The structure of the FOXC1 gene

The *FOXC1* gene encodes the functional protein product FOXC1 (formerly known as 'FREAC3' and 'FKHL7'), containing 553 amino acid residues.[11,39] Previous studies have identified that FOXC1 possesses important functional regions from the N- to C-terminal boundary required for nuclear localization and transcriptional regulation.[40] Followed by the activation domain 1 (AD1) at the N-terminal, the forkhead domain (FHD), the inhibitory domain/phosphorylation domain, and the activation domain 2 (AD2) are arranged in sequence. The two transcription activation domains, AD1 and AD2, lie at positions 1–51 and 466–553, respectively. The N-terminal activation domain (AD1) is more active in the context of FOXC1 than in GAL4, a yeast transcription activator protein used to study gene expression, and therefore may be responsible for activation of FOXC1-specific target genes.[40] However, the C-terminal activation domain (AD2) may serve as a general transcriptional activator capable of activating transcription.[40,41]

The FHD exists as an approximately 110 amino acid segment that is commonly shared by FOX proteins.[4,42] Owing to the FHD element, FOX proteins have capacity to bind to DNA. Despite the high conservation of amino acid sequences in the FHD, there is little amino acid similarity in the activation domains between FOXC1 and other FOX proteins. The FHD of *FOXC1* contains nuclear localization signal (NLS) at the N- and C-terminal responsible for FOXC1 translocation to cell nucleus.[40,43] The first region required for FOXC1 nuclear localization spans from residues 77 to 93 in the FHD, but this amino acid sequence does not match any typical NLS motif.[44] The second region contains a basic stretch of amino acids at position residues 169–176 at the C-terminal of the FHD, which is similar to the NLS found in a number of HOX proteins.[45] Only the C-terminal region of the FHD, rich in basic residues, represents a *bona fide* NLS.[40,46] Thus, missense mutations in FHD can significantly reduce protein stability, transactivation ability, and DNA binding capacity of FOXC1.[43]

According to the current studies, the structure of the *FOXC1* gene is significantly different from other homologous members in the FOX family. Because the expression and regulation of FOXC1 is tightly associated with its gene functional regions, more precise studies are needed to clarify its detailed gene segments.

Regulatory mechanisms of FOXC1

FOXC1 expression can be controlled at multiple levels, including modulation of DNA transcription, post-transcriptional regulation, and post-translational modifications. To further understand the molecular mechanisms involved in regulating FOXC1 expression and activity, we will discuss the multiple types of regulation of FOXC1 in physiological or pathological conditions in this section (Table 2).

Modulation of DNA transcription

Epigenetic alterations play key roles in the silencing of target genes through DNA methylation and histone modification processes. The methylation rate of the *FOXC1* promoter region is significantly increased in invasive breast cancer compared with adjacent normal tissues, which may downregulate FOXC1.[60,61] Remarkably, transcription of *FOXC1* gene has been reported recently to be repressed by endogenous enhancer of zeste homologue 2 (EZH2) through methylation of histone H3 at lysine 27 (H3K27) and H3/H4 acetylation of *FOXC1* promoter in breast cancer.[47] EZH2 is a member of the polycomb group (PcG) of proteins, which have been demonstrated as epigenetic silencers in cancer development. It is also a histone methyltransferase involved in transcriptional repression.[62,63] The continued

Table 1. Overview of FOXC1 function as reported in different types of cancer.

Cancer categories	Expression of FOXC1	Subcellular localization in tumor cells	Correlation between the FOXC1 expression level with clinicopathological features of tumors	Effect of overexpression of FOXC1	Study
BLBC	mRNA and protein are higher	Both in the nucleus and in the cytoplasm	Elevated FOXC1 expression is positively associated with brain metastasis and inversely associated with bone metastasis.	Increase cell proliferation, EMT migration, and invasion	Ray *et al.*[16] Ray *et al.*[8]
Ovarian cancer	Protein is higher	Mainly in the nucleus and less in the cytoplasm	There is a trend for positive FOXC1 expression to decrease with advancing FIGO stage and pathological subtypes from benign to malignant tumors.	–	Wang *et al.*[19]
EEC	mRNA and protein are higher	–	–	Promote cell growth and migration and suppress apoptosis	Xu *et al.*[22]
Cervical carcinoma	mRNA and protein are higher	Both in the nucleus and in the cytoplasm	There is a positive correlation between FOXC1 expression and tumor stage, tumor size, stromal invasion, and lymph nodes metastasis.	Facilitate cell proliferation and migration	Huang *et al.*[23]
Prostate cancer	mRNA is lower	–	There is no significant evidence between FOXC1 expression and clinicopathological features.	Inhibit invasive progression	van der Heul-Nieuwenhuijsen *et al.*[24]
HCC	mRNA and protein are higher	Primarily in the nucleus	Upregulation of FOXC1 is positively related with tumor size, microvascular invasion, the degree of tumor differentiation, and TNM stage.	Promote HCC cell invasion and metastasis through induction of EMT	Xia *et al.*[17]
PDA	mRNA and protein are higher	–	FOXC1 upregulation in patients with advanced clinical stage, poor histological differentiation, and present lymph node metastasis.	–	Wang *et al.*[21]
Gastric cancer	mRNA and protein are higher	Predominantly in the nucleus	FOXC1 positivity correlates with the degree of histological differentiation, TNM stage, invasive depth, lymph node metastasis and distant metastasis.	–	Xu *et al.*[25]
ESCC	mRNA and protein are higher	–	FOXC1 upregulation tends to show higher TNM stage and lymph node metastasis, and poor survival status of ESCC patients.	Enhance tumor cell proliferation, migration and invasion	Pan *et al.*[26] Zhu *et al.*[27]
OSCC	mRNA is higher	–	There is no significant correlation between FOXC1 expression and clinicopathological features.	Increase the cell proliferation and migration ability of OSCC	Kong *et al.*[28]
LSCC	mRNA and protein levels are higher	–	Higher FOXC1 expression is associated with high TNM stage.	Enhance cell proliferation, colony formation, migration and invasion	Gao *et al.*[29]

Table 1. (Continued)

Cancer categories	Expression of FOXC1	Subcellular localization in tumor cells	Correlation between the FOXC1 expression level with clinicopathological features of tumors	Effect of overexpression of FOXC1	Study
Tongue cancer	mRNA is higher	–	FOXC1 expression is positively related with lymph node metastasis and the clinical stage of TSCC.	Increase EMT	Lin *et al.*[30]
Nasopharyngeal carcinoma	Protein is higher	Both in the nucleus and in the cytoplasm	High expression of FOXC1 positively correlates with tumor size, lymph node metastasis, distant metastasis and clinical stage.	Enhance tumor growth and EMT	Ou-Yang *et al.*[20] Liu *et al.*[31]
NSCLC	mRNA and protein are lower	Primarily in the nucleus and less in the cytoplasm	FOXC1 overexpression is significantly correlated with poor tumor differentiation, high TNM stage, and lymph node metastasis.	FOXC1 silencing inhibits NSCLC cell proliferation and migration.	Chen *et al.*[32] Wei *et al.*[18]
AML	mRNA is higher	–	High FOXC1 expression significantly indicates different morphologic classifications, including FAB-M2, M4, and M5.	Maintain clonogenic potential of AML cells, and block differentiation of AML cells and monocytic lineage	Somerville *et al.*[33]
Hodgkin lymphoma	Protein is lower	–	–	Inhibit B-cell apoptosis	Nagel *et al.*[34]
DLBCL	protein is higher	–	–	Promote invasion and metastasis	Blonska *et al.*[35]
Melanoma	mRNA and protein are lower	–	FOXC1 up-regulation is positively correlated with AJCC stages.	Promote proliferation, colony formation, migration and invasion of melanoma cell	Wang *et al.*[36]
RCC	mRNA is higher	–	–	–	Yao *et al.*[37]
Cholangiocarcinoma	protein is higher	–	–	–	Li *et al.*[38]

AML, acute myeloid leukemia; BLBC, basal-like breast cancer; DLBCL, diffuse large B-cell lymphoma; EEC, endometrioid endometrial cancer; EMT, epithelial-to-mesenchymal transition; ESCC, esophageal squamous cell carcinoma; FOXC1, forkhead box C1; HCC, hepatocellular carcinoma; LSCC, laryngeal squamous cell carcinoma; NSCLC, non-small cell lung cancer; OSCC, oral squamous cell carcinoma; PDA, pancreatic ductal adenocarcinoma; RCC, renal cell carcinoma; –, unavailable in the original article.

Table 2. Summary of regulatory mechanisms and activity alterations of FOXC1.

Regulatory factor	Regulatory mechanism	Effect on FOXC1	Tumor	Study
EZH2	Methylates H3K27 and acetylates H3/H4 of FOXC1 promoter	Represses the expression of FOXC1	BLBC AML	Du *et al.*[47] Somerville *et al.*[33]
BRCA1	Binds to the FOXC1 distal promoter	Represses the expression of FOXC1	BLBC	Tkocz *et al.*[48]
miR-204 miR-495	Binds to 3'-untranslated regions of FOXC1 mRNA	Represses the expression of FOXC1	EEC EEC	Chung *et al.*[49] Xu *et al.*[22]
miR-204-5p	Binds to 3'-untranslated regions of FOXC1 mRNA	Represses the expression of FOXC1	LSCC	Gao *et al.*[29]
miR-639	Binds to 3'-untranslated regions of FOXC1 mRNA	Represses the expression of FOXC1	Tongue cancer	Lin *et al.*[30]
miR-4792	Binds to 3'-untranslated regions of FOXC1 mRNA	Represses the expression of FOXC1	NPC	Li and Chen[50]
miR-138-5p	Binds to 3'-untranslated regions of FOXC1 mRNA	Represses the expression of FOXC1	Pancreatic cancer	Yu *et al.*[51]
miR-374c-5p	Binds to 3'-untranslated regions of FOXC1 mRNA	Represses the expression of FOXC1	Cervical cancer	Huang *et al.*[52]
miR-133	Binds to 3'-untranslated regions of FOXC1 mRNA	Represses the expression of FOXC1	Glioma	Liu *et al.*[53]
miR-133	Binds to 3'-untranslated regions of FOXC1 mRNA	Represses the expression of FOXC1	Pituitary adenoma	Wang *et al.*[54]
FOXCUT	Binds to FOXC1 mRNA	Upregulates the expression of FOXC1	BLBC OSCC ESCC NPC	Kong *et al.*[28] Liu *et al.*[55] Pan *et al.*[26] Xu *et al.*[56]
ERK1/2	Phosphorylates FOXC1 at Ser-272	Enhances the expression and activity of FOXC1	HCC BLBC	Berry *et al.*[57]
Akt	Phosphorylates FOXC1 protein	Enhances the expression and activity of FOXC1	BLBC	Jin *et al.*[58]
Ubiquitin-26 S proteasome	Polyubiquitinates FOXC1 protein	Induces degradation of FOXC1	–	Berry *et al.*[57]
SUMO2/3	SUMOylates FOXC1 protein	Inhibits the activity of FOXC1	–	Danciu *et al.*[59]

Akt, protein kinase B; AML, acute myeloid leukemia; BLBC, basal-like breast cancer; BRCA1, breast cancer susceptibility gene 1; EEC, endometrioid endometrial cancer; ERK1/2, extracellular signal-regulated kinase 1/2; ESCC, esophageal squamous cell carcinoma; EZH2, enhancer of zeste homologue 2; FOXC1, forkhead box C1; miR, microRNA; FOXCUT, FOXC1 promoter upstream transcript; HCC, hepatocellular carcinoma; LSCC, laryngeal squamous cell carcinoma; NPC, nasopharyngeal carcinoma; OSCC, oral squamous cell carcinoma; SUMO, small ubiquitin-like modifier; –, unavailable to date.

repression of *FOXC1* through methylation by EZH2 can also be observed in normal human CD34+ hematopoietic stem and progenitor cells.[33] Correspondingly, loss of EZH2 activity may contribute to the derepression of FOXC1 in acute myeloid leukemia (AML).[33] Recently, Wang and colleagues[36] detected an elevated level of FOXC1 protein in M219 and M15 melanoma cells after treatment with a 5-Aza-demethylation agent. Therefore, hypermethylation of *FOXC1* results in decreased expression of FOXC1 in melanoma. Further investigations are required to verify the functional consequence of increased DNA methylation and histone modifications in the regulation of FOXC1 during the progression from normal tissues to invasive carcinoma.

In addition, FOXC1 was reported to be transcriptionally repressed by a tumor suppressor gene, breast cancer susceptibility gene 1 (BRCA1) in BLBC.[48] BRCA1 can partake in transcriptional regulations through interactions with sequence-specific transcription factors.[64,65] After BRCA1 binds to the FOXC1 distal promoter, GATA3 (a crucial regulator of luminal differentiation in breast tissue) is required to bind to the C-terminal of BRCA1 and, consequently, BRCA1 exerts an inhibitory effect on the expression of FOXC1.[48]

Post-transcriptional regulation

The post-transcriptional regulation by endogenous small noncoding RNA, especially microRNA (miRNA), and long noncoding RNA (lncRNA) may be a potential method for regulating gene expression in human cancer. MiRNAs are a series of 18- to 25-nucleotide non-coding RNAs that inhibit protein translation through sequence-specific pairing with 3'-untranslated regions (3'-UTR) of target mRNA.[66] In endometrioid endometrial cancer (EEC) cell lines, FOXC1 protein level is directly downregulated by overexpression of miR-204 and miR-495.[22,49] Recently, it has been reported that miR-204-5p has a negative regulatory effect on FOXC1 expression in laryngeal squamous cell carcinoma (LSCC).[29] In addition, miR-374c-5p can repress FOXC1 expression in cervical cancer by directly targeting the FOXC1 3'-UTR.[52] In tongue squamous cell carcinoma, a reporter assay with the 3'-UTR of *FOXC1* cloned downstream of the luciferase gene exhibited decreased luciferase activity in the presence of miR-639, indicating that miR-639 is a direct inhibitor of *FOXC1* expression.[30] In addition, reduced activity and expression of FOXC1 have been demonstrated in the presence of up-regulated miR-4792 and miR-138-5p in NPC[50] and pancreatic cancer,[51] respectively. Interestingly, miR-133 can exert its tumor-suppressive function through directly targeting and inhibiting FOXC1 expression in both glioma[53] and invasive pituitary adenoma.[54] Based on cell line studies *in vitro*, all these miRNAs have bene shown to favor cancer development and progression *via* direct inhibition of FOXC1 expression.

LncRNAs exist as novel RNA transcripts with no protein-coding potential that are longer than 200 bases in length, which function as high-level regulators involved in complementary base pairing with target mRNA in post-transcriptional processes.[67] A novel lncRNA named *FOXC1* promoter upstream transcript (FOXCUT) has been identified to functionally contact its adjacent FOXC1 mRNA and take on the form of 'lncRNA-mRNA pairs' in esophageal squamous cell carcinoma (ESCC), oral squamous cell carcinoma (OSCC), BLBC, and NPC.[26,28,55,56] High levels of FOXCUT may positively increase the mRNA levels of FOXC1. Consistently, RNA interference analysis revealed that knockdown of FOXCUT remarkably attenuated the level of FOXC1 mRNA expression, which was in accord with inhibited cancer cell growth rates and metastatic capability.

Post-translational modifications

The transcriptional inhibitory domain located at amino acids 215–366 of FOXC1 contributes to a phosphorylation-dependent mobility alteration in the FOXC1 protein.[40] Removal of this region disputes this mobility shift and leads to a transcriptionally hyperactive form of FOXC1. Extracellular signal-regulated kinase 1/2 (ERK1/2) contributes to FOXC1 phosphorylation and transcriptional activation in response to epidermal growth factor (EGF), which is dependent upon Ser-272 of FOXC1.[57] ERK1/2 is integral in controlling activity and stability of FOXC1, as repression of ERK1/2 activity by pharmacological manner or the elimination of serum growth factors can decrease the stable state levels of FOXC1 protein. Interestingly, hepatitis B virus X can increase FOXC1 expression and phosphorylation by promoting the binding of ERK1/2 to the promoter of FOXC1 in HCC cell lines.[17] In addition, EGF potently increases FOXC1 expression and phosphorylation through Ras/ERK and phosphatidylinositol 3-kinase (PI3K)/Akt pathways in BLBC cells.[58] Inhibition of ERK and PI3K by the respective small-molecule inhibitors U0126 and LY294002 exerts a more pronounced inhibitory effect on the EGF-mediated increase in FOXC1 activity and expression levels.[58] Noticeably, the EGFR activation can promote nuclear translocation of NF-κB through Ras/ERK and PI3K/Akt pathways, which binds to the FOXC1 promoter and consequently increases FOXC1 expression.[68] Even so, further investigations are required to define the complete mapping of phosphorylation sites of FOXC1.

Ubiquitination acts as a signal for the degradation of FOXC1, which may characterize FOXC1 with a short-lived transcription factor.[57] FOXC1 is polyubiquitinated and degraded *via* the

ubiquitin-26 S proteasome manner, which occurs at the 367–553 residue region. Moreover, the phosphorylated residue Ser-272 by ERK1/2 may impede the interaction between an E3-ubiquitin ligase with the C-terminus of FOXC1.[57] However, future investigations should be aimed at verifying the molecular counterparts that can modulate the ubiquitination and degradation of FOXC1, which is crucial for understanding of FOXC1 activity and stabilization.

In addition, small ubiquitin-like modifier (SUMO) modifications have been established as a critical type of modification that exert significant inhibitory effects on FOXC1 activity. The mammalian SUMO family is composed of four members designated SUMO1–4, whereas endogenous FOXC1 is demonstrated to be mainly modified by SUMO2 and SUMO3.[59,69] SUMO can be activated and transferred to a substrate *via* ubiquitin enzymes following SUMO-specific protease-mediated C-terminal proteolytic process, thus consequently forming an isopeptide bond between the C-terminus of SUMO and the amino radical of the target lysine.[70,71] SUMOylation of FOXC1 occurs primarily on lysine sites in one consensus synergy control motif with less contributions of a second, more degenerate motif,[59] both of which cooperate functionally. Importantly, SUMOylation-deficient mutants showed increased transcriptional activity of FOXC1 compared to wild-type forms, although they exhibited similar protein levels and subcellular localization.[59]

Taken together, FOXC1 activity and expression levels can be regulated in various aspects of gene expression. However, all the detailed regulatory mechanisms have not been fully uncovered. Further, whether these regulatory actions exert negative or positive effects on cancer development accompanied by abnormal expression and activity of FOXC1 requires additional investigation.

FOXC1 expression in cancer

The expression levels of FOXC1 mRNA and protein in cancerous tissues have been demonstrated to be congruously higher than those in non-cancerous tissues, such as BLBC,[8] HCC,[17] NSCLC,[32] gastric cancer (GC),[25] and NPC[31] (Table 1). Steady overexpression of FOXC1 can lead to alterations in the expression of target genes, which are indicative of evasion of apoptosis, epithelial-to-mesenchymal transition (EMT), and increased cellular migration and invasion. Importantly, FOXC1 may act as a specific marker for subtypes of breast cancer and AML. It was observed by Ray and colleagues[8] that FOXC1 expression is markedly higher in BLBC than in luminal and HER2 breast cancer, indicating that detection of FOXC1 can be utilized as a specific biomarker for basal-like subtype in breast cancer classifications. High levels of FOXC1 in AML (FAB-M2) exhibit essential relevance with morphological classifications associated with granulocyte differentiation compared with other AML subtypes.[33] Notably, elevated FOXC1 expression is also considered to be a potential marker of disease relapse and failure during induction chemotherapy in AML,[72] suggesting the diagnostic and predictive utility of FOXC1 expression in the clinical management of AML. In addition, in most cancer types, high FOXC1 expression may predict clinical features such as malignant clinical manifestations, increased pathological grade, and poor outcomes of patients.

In addition to the differences in FOXC1 expression levels, the intracellular location of FOXC1 may vary from noncancerous tissues to cancerous tissues. FOXC1 localizes totally to the nucleus in almost all wild-type cells depending upon its NLS located at the C-terminus of the FHD.[39,73] When analyzing the expression of FOXC1 in cancer cells, including NSCLC, NPC, and breast cancer cells, localization of FOXC1 in both the nucleus and the cytoplasm of cells is always observed.[18,25,31] This incomplete localization may be due to a deficiency in the capability of FOXC1 to be retained within the nucleus or, alternatively, may arise from inefficient transport of defective FOXC1 molecules into the nucleus.[73] These disparate expression patterns might arise from discrepant oncogenic environmental stimuli or diverse abnormal regulation of FOXC1. However, to date, no explicit mechanism has been elucidated whereby FOXC1 is distinguishingly localized in cancer cells.

FOXC1-targeted processes in tumors

The pleiotropic functions of FOXC1 in cancer have been identified in various pathological processes, including aberrant cell proliferation, cancer stem cell (CSC) maintenance, tumor migration, and potential angiogenesis. The key FOXC1 target genes and pathways underlying these processes have been reviewed accordingly (Figure 1).

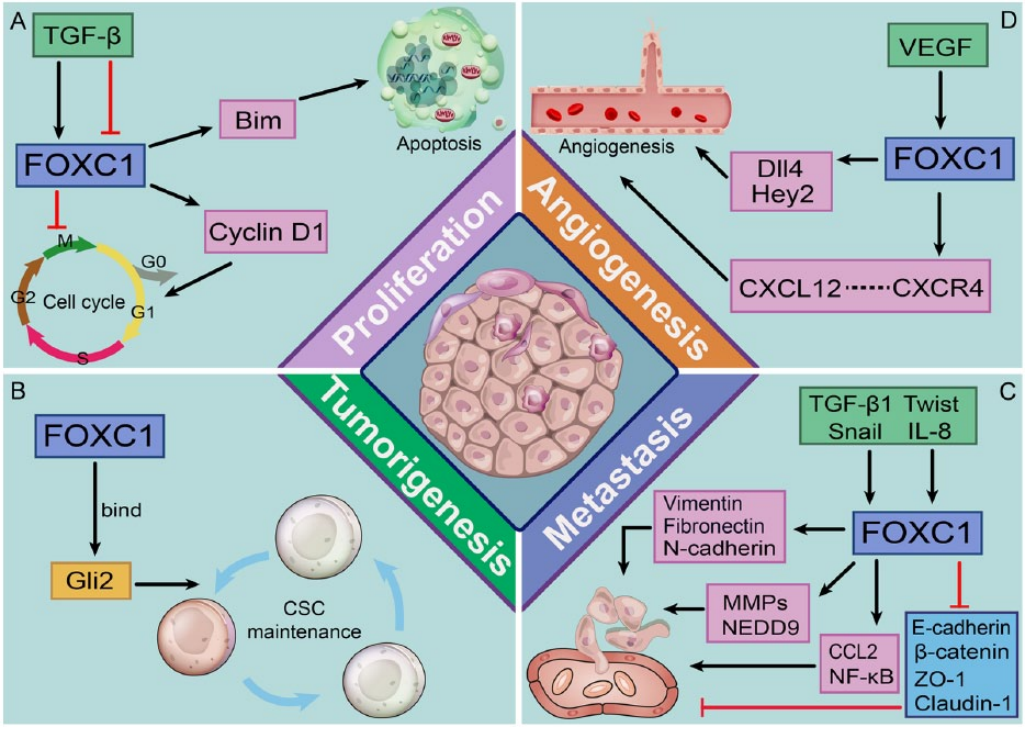

Figure 1. Overall actions of FOXC1 on the hallmarks of tumor biology. A. FOXC1 acts as a downstream target of TGF-β. On one hand, FOXC1 can be activated by TGF-β to suppress cell proliferation by inducing cell cycle arrest. On the other hand, TGF-β downregulates FOXC1 and, consequently, inhibits cell apoptosis by reducing the expression of the pro-apoptotic protein Bim, which is an essential target of FOXC1. Moreover, FOXC1 augments the expression of cyclin D1 to promote cell proliferation involved in tumorigenesis. **B.** Binding of FOXC1 to Gli2 induces CSC maintenance to promote tumorigenesis. **C.** FOXC1 acts as a master regulator of metastasis. FOXC1 can be upregulated by TGF-β1, Snail, and Twist and is activated by IL-8. FOXC1 significantly increases the expression of its various downstream targets to promote cancer metastasis, including several mesenchymal markers (vimentin, fibronectin, and N-catenin), inflammation-related cytokines (CCL2 and NF-κB), and other important pro-metastatic proteins (MMPs and NEDD9). FOXC1 also inhibits the expression of several epithelial markers to facilitate EMT, such as β-catenin, E-cadherin, ZO-1, and claudin-1. **D.** VEGF may activate FOXC1 and thus increase FOXC1-mediated activation of Dll4 and Hey2, which leads to tumor angiogenesis. In addition, activation of FOXC1 induced by VEGF can increase CXCR4 expression and consequently promote angiogenesis through enhanced CXCR4-CXCL12 pathway.
Bim, Bcl-2 interacting mediator of cell death; Dll4, Delta-like 4; CCL2, chemokine (C-C Motif) ligand 2; CXCR4, CXC chemokine receptor type 4; CXCL12, CXC chemokine ligand 12; FOXC1, forkhead box C1; IL-8, interleukin-8; MMP, matrix metalloproteinase; NEDD9, developmentally down-regulated protein 9; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.

FOXC1 in cell proliferation and apoptosis

Regulation of apoptotic pathways appears to serve a critical juncture for the control of unbridled cell proliferation and tumor growth. Intriguingly, the role that FOXC1 plays in the regulation of the cell cycle leading to anti-apoptosis or pro-apoptosis remains controversial in tumor progression.

The antitumor effects of FOXC1 have been identified to be closely related to the inhibition of proliferation. Petrini and colleagues[74] reconstituted FOXC1 into FOXC1-negative osteosarcoma U2OS cells and showed that ectopic FOXC1 attenuates anchorage-independent growth and motility of U2OS cells *in vitro*. Similarly, they also observed FOXC1-mediated cell growth inhibition in TET cells.[74] The results in both U2OS and TETs cells suggest the potential antitumor activity of FOXC1. In addition, FOXC1 acts as a downstream target of transforming growth factor-β (TGF-β) to block tumorigenesis. Proteins of the TGF-β superfamily, which comprise three different isoforms (TGF-β1, TGF-β2, and TGF-β3), are multifunctional cytokines that elicit transcriptional responses to many target genes to mediate their diverse effects on control of the cell cycle to either inhibit or promote apoptosis.[75] Of note, upregulated

transcription of FOXC1 induced by TGF-β1 has been observed in several human cancer cell lines, including ovarian cancer cell lines (HAC1 and SKOV3), an endometrial cancer cell line (HHUA), and a cervical cancer cell line (SiHa).[9] Furthermore, increased FOXC1 makes cells more sensitive to TGF-β1-mediated growth inhibitory effects. In addition, ectopic expression of FOXC1 can restore the potential of TGF-β1 to impede cell growth by arresting cells in the G_0/G_1 phase in FOXC1 homozygously deleted HeLa cells.[9] However, the mechanisms and downstream targets underlying this TGF-β1-FOXC1-induced cell cycle arrest remain unclear. Moreover, FOXC1 is also upregulated by TGF-β1 in EMT and, consequently, increases the expression of fibroblast growth factor receptor 1 (FGFR1) in order to control mesenchyme cell fate decisions and promote an invasive activated fibroblast phenotype.[76] Intriguingly, in breast cancer cells, the expression of FOXC1 is regulated by TGF-β in the complete opposite direction. Hoshino and colleagues[77] found that endogenous TGF-β promotes the survival of breast cancer cell lines, JygMC(A) and JygMC(B), by downregulating FOXC1 mRNA and its downstream target, Bcl-2 interacting mediator of cell death (Bim), which is a pro-apoptotic protein in cancer cells. Notably, these discrepancies in FOXC1 expression and effects on tumor cell growth may result from the differences in the cell types tested and experimental conditions. Furthermore, although TGF-β has been generally accepted to play a dual role in tumorigenesis,[78] the interaction between TGF-β and FOXC1 and signaling downstream of FOXC1 in cancer cell apoptosis remain undefined.

Some compelling evidence from the studies in breast cancer points towards an oncogenic role of FOXC1. Ectopic FOXC1 overexpression in MDA-MB-231 BLBC cells contributed to increased tumor cell proliferation.[8] Similarly, MCF-7 luminal breast cancer cells with FOXC1 overexpression exhibited enhanced anchorage-independent growth in soft agar. In contrast, shRNA-mediated FOXC1 knockdown in 4T1 mouse breast cancer cells, a model for stage IV human breast cancer that possesses high levels of endogenous FOXC1, yielded the opposite effects, that is, it suppressed cell proliferation and decreased tumorigenic ability.[8,79] Furthermore, the aberrant promotion of cellular proliferation in breast cancer cells may arise from the upregulation of cyclin D1 induced by FOXC1 overexpression. In normal cells, cyclin D1 plays an essential rate-limiting role at the G_1/S phase restraining aberrant cell proliferation. However, in cancer cells, cyclin D1 is activated and highly expressed without being degraded at the S phase, leading the cell cycle out of control and ultimately to tumorigenesis.[8,80] Therefore, FOXC1 might be involved in the promotion of tumorigenesis by augmenting the expression of cyclin D1. In addition, the same interaction and effect of FOXC1 with cyclin D1 was observed in NSCLC A549 and NCIH460 cells by Chen and colleagues.[32]

In general, FOXC1 contributes to tumorigenesis by promoting cell proliferation. However, FOXC1 can exert antiproliferative effects to suppress tumor growth. These contradictory results might be explained by cellular context-dependent and/or cell-type-dependent effects of FOXC1 despite the fact that the cells derive from the same type of tissue. Moreover, the divergent study designs and experimental materials, for example, the different choices and titer of antibodies in immunohistochemistry (IHC) or immunoblotting, might also be partly responsible for the inconsistent phenomena. Therefore, further investigations are imminently required to elucidate more accurate details of FOXC1-mediated proliferation and apoptosis in cancer cells.

FOXC1 in cancer stem-like cells

CSCs have been identified to play critical roles in tumor growth, migration, and relapse. Han and colleagues[81] injected FOXC1-knockdown BT549 cells into mouse mammary glands and then observed decreased tumor size. *In vitro*, overexpressed FOXC1 in SUM159 and MDA-MB-468 cells remarkably enhanced aldehyde dehydrogenase activity, the increase of which is used for characterizing breast CSC. Both results indicate that FOXC1 positively regulates CSC properties of BLBC cells *in vivo* and *in vitro*. FOXC1 regulates CSC maintenance through activation of Smoothened (SMO)-independent hedgehog (Hh) signaling in BLBC cells. Hh signaling is one of the classical signaling pathways involved in normal stem cell function and CSC maintenance.[82] Binding of FOXC1 to Gli2, an ultimate downstream molecule of Hh, is specifically required for the activation of Hh signaling.[81,83] Therefore, inactivation of Gli2 and blockage of the Hh pathway might be potential ways to attenuate FOXC1-induced tumorigenicity.

FOXC1 in cell invasion and metastasis

Cancer metastasis to distant organs is mainly characterized by activation of the EMT pathway, a process by which polarized epithelial cells acquire mesenchymal properties, including increased potential for motility and metastasis.[84] The EMT process is characterized by remarkably enhanced expression of several mesenchymal markers, including N-catenin, vimentin and fibronectin, and by translocation or reduced expression of some epithelial markers such as β-catenin, E-cadherin, zonula occludens-1 (ZO-1), and claudin-1.[84] As FOXC1 is not sufficient to induce the entire EMT process on its own, it has been identified that FOXC1 functions downstream of TGF-β1, Snail, and Twist, all of which are the most potent EMT-inducers present in tumor microenvironments. FOXC1 is consistently elevated by overexpression of TGF-β1, Snail, and Twist in EMT.[84–87] Notably, the dual effects of TGF-β as both a tumor suppressor and a prometastatic mediator make it an interesting target for investigating the implications of FOXC1 intervention at the crossroad of proliferation and metastasis. Suppressed FOXC1 expression in human HCC Bel-7402 cells and SK-Hep1 cells reverses EMT progress by downregulating vimentin and N-catenin, translocating β-catenin to the cytoplasm, and increasing the expression of ZO-1 and claudin-1.[86] In addition, attenuated expression of E-cadherin and augmented expression of vimentin and fibronectin was observed in human HCC SMMC7721 cells with increased metastatic potential induced by enhanced FOXC1 levels.[17] Downregulation of E-cadherin also serves as a hallmark of EMT in ESCC, in which FOXC1 may promote EMT through activating Zinc finger E-box-binding homeobox 2 (ZEB2), a well-reported transcriptional suppressor of E-cadherin.[27] In invasive NPC tissues, high expression levels of FOXC1 upregulate mesenchymal traits, including vimentin, fibronectin, and N-cadherin, although without altering the expression of β-catenin and E-cadherin.[20] These results suggest that FOXC1 may contribute to EMT by enhancing cell-extracellular matrix adhesion rather than disrupting adherens junctions in NPC, whereas it exerts both effects in HCC. The inconsistency may arise from the specificities of different tissues. Interestingly, a contradictory phenomenon that attenuated invasion and metastasis in response to higher FOXC1 expression was observed by Du and colleagues in MDA-MB-231HM (high metastasis) cells, in which FOXC1 may repress vimentin expression.[47] These sharply inconsistent outcomes of FOXC1 in regulating EMT-related genes might be attributed to different cell materials or tumor microenvironments. Moreover, reduced FOXC1 expression was reported in MCF-7 breast cancer cells to activate the mesenchymal-to-epithelial transition (MET) pathway, a process through which tumor cells reactivate certain epithelial properties at the secondary neoplastic site.[88]

In addition, FOXC1 has also been implicated in inflammation-related tumor metastasis. Pro-inflammatory cytokine interleukin-8 (IL-8) can activate FOXC1 through activation of hypoxia-inducible factor 1α (HIF-1α) by the PI3K/Akt pathway. Consequently, activated FOXC1 transactivates chemokine (C-X-C Motif) receptor 1 (CXCR1), which is a crucial promoter of cancer cell motility through activation of Rho-GTPases, elevates invasion and metastasis in HCC.[89,90] In addition, chemokine (C-C Motif) ligand 2 (CCL2), which is upregulated in several human cancer and regulates macrophage motility and migration, was identified to be directly activated by FOXC1 in Huh7-FOXC1 cell lines and HCCLM3-shFoxC1 cell lines.[89,91] This transactivation of CCL2 by FOXC1 significantly promoted macrophage infiltration and cancer metastasis in HCC mouse models. Moreover, Wang and colleagues[92] found that augmented FOXC1 attenuates the ubiquitination and degradation of nuclear factor kappa light chain enhancer of activated B cells (NF-κB) to promote aggressive cellular traits, including invasion and metastasis, commonly associated with BLBC.

FOXC1 can also increase cell invasion by induction of the matrix metalloproteinase (MMP) family members. Silencing endogenous FOXC1 in HCC1187 human basal breast cancer cells leads to reduced MMP7 expression and decreased viability and motility of these cancer cells.[93] In FOXC1-delepted HCC cells, MMP1, MMP2, MMP7, and MMP9 were observed to be simultaneously downregulated upon FOXC1 suppression.[86] Therefore, inhibiting MMPs in the increased aggression imparted by FOXC1 overexpression may be an efficient strategy to prevent distant metastasis of a malignant tumor. In addition, Xia and colleagues[17] conducted a detailed compare of gene expression between HCCLM3-shFoxC1 cells and HCCLM3-sh control cells and found that developmentally downregulated protein 9 (NEDD9) is downregulated 8.7-fold in neural precursor cells in response

to the knockdown of FOXC1. NEDD9 acts as a scaffolding protein that promotes integrin-dependent migration and invasion in cancer.[94] FOXC1 binds directly to the NEDD9 promoter in HCC cells and subsequently enhances tumor metastasis. However, further investigations of the associations between FOXC1 and NEDD9 in other different types of cancer are required.

In conclusion, FOXC1 operates as a master metastasis regulator of EMT and several prometastatic factors. Further studies should determine how these data can be used to develop a better treatment by targeting these mechanisms for suppressing cancer metastasis.

FOXC1 in tumor angiogenesis

Vascular endothelial growth factor (VEGF) signaling precisely coordinates and sequentially activates the Delta–Notch pathway to promote angiogenesis.[95,96] FOXC1 directly activates the Delta-like 4 (Dll4) promoter, which acts as an upstream regulator of Notch signaling in arterial specification, and induces its transcription.[97] Except for the induction of Dll4 expression, Hey2, as a target gene of Notch, can also be transcriptionally activated by FOXC1.[97] VEGF signaling may augment FOXC1-induced activation of Dll4 and Hey2 through phosphorylation of FOXC1.[97,98] Collectively, FOXC1 interacts with VEGF and Notch signaling to augment arterial gene expression in multiple steps of the Delta–Notch signaling pathway and, therefore, participates in tumor angiogenesis.

The CXC chemokine receptor type 4 (CXCR4)-CXC chemokine ligand 12 (CXCL12) pathway has been significantly implicated in the progression of angiogenesis such as *via* endothelial cell migration and capillary tube formation.[99] FOXC1 has been reported to markedly transactivate the CXCR4 promoter and to increase the expression of CXCR4 in endothelial cells. Consistently, attenuation of CXCR4 expression and CXCL12-induced cell migration were observed in FOXC1-deficient vascular endothelial cells.[100] Moreover, under certain conditions, the CXCR4–CXCL12 pathway activated by FOXC1 has the capability to promote the growth of pre-existing blood vessels and recruit CXCR4+ endothelial progenitor cells (EPCs) from bone marrow to the neo-angiogenic niches supporting revascularization of tumor growth.[101,102] Salcedo and colleagues[103] reported that the VEGF pathway can induce CXCR4 expression in endothelial cells through indirect activation of FOXC1 in arterial gene expression. However, there is not adequate convincing evidence on the interaction between FOXC1 and tumor angiogenesis in explicit cancer types. The results mentioned above may provide novel directions for further studies of FOXC1 in tumor angiogenesis.

Interestingly, EMT has been identified as promoting angiogenesis *via* upregulation of VEGF-A.[104] Moreover, a high angiogenic potential induced by EMT and the increased VEGF-A can result in the enhanced CSC tumorigenicity.[104] Notably, significantly decreased VEGF-A expression was observed after FOXC1 silencing in HCC cells.[86] As FOXC1 plays a vital role in regulating EMT progress and CSC properties, FOXC1 may increase angiogenesis partly by modulating EMT programs and consequently support microvascular invasion to favor cancer metastasis. Meanwhile, the increased tumorigenicity of CSCs might be induced by potential FOXC1-EMT and/or FOXC1-VEGF signaling for tumor growth. However, the mechanisms of FOXC1 involvement in these possible interactions require more investigations.

FOXC1 application in cancer therapy

Recent studies have suggested that FOXC1 may play paradoxical roles in cancer therapy (Figure 2). FOXC1 overexpression renders cancer cells more susceptible to some pharmacologic interventions. On the other hand, increased FOXC1 expression can also be involved in drug resistance.

FOXC1 and drug response

Comparing three BRCA1-mutant BLBC cell lines (SUM1315, SUM149, and MDA-MB-436) with one BRCA1 wild-type cell line (BT549), Johnson and colleagues[105] found that higher expression of FOXC1 in BRCA1-mutant cell lines induced sensitivity to 10 μM olaparib, a PARP inhibitor utilized in BRCA-mutant cancer that takes advantage of synthetic lethality with repair defect, whereas lower expression of FOXC1 in BT549 cell did not affect olaparib treatment. In addition, a case reported by Wang and colleagues[92] identified that overexpression of FOXC1 had the ability to sensitize MDA-MB-231 cells to pharmacologic NF-κB inhibitors BMS-345541 and BAY-117082, leading to prominently decreased cell proliferation,

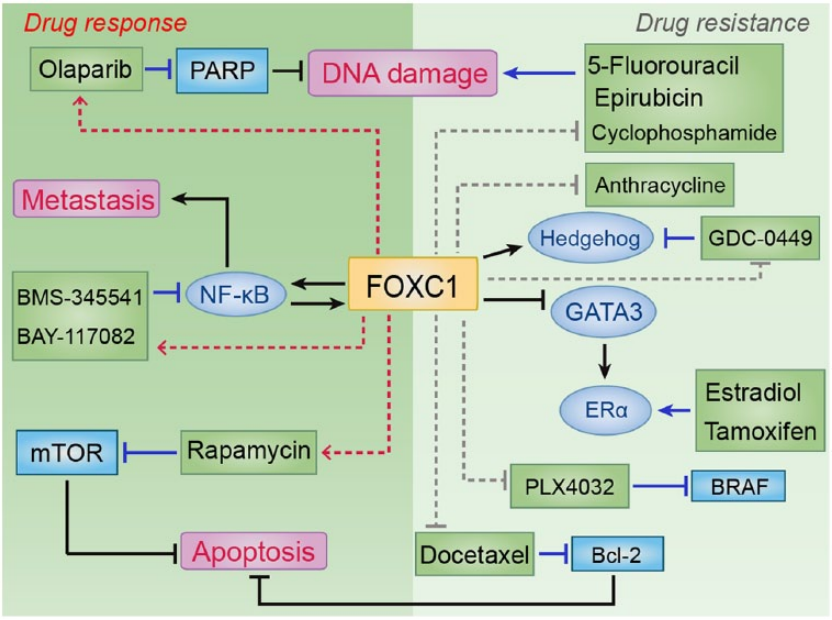

Figure 2. Schematic representation of drug response and drug resistance involving FOXC1 or FOXC1-dependent pathways. Overexpression of FOXC1 induces the sensitivity of BLBC cells to a PARP inhibitor, olaparib. FOXC1 can also sensitize breast cancer cells to the NF-κB inhibitors BMS-345541 and BAY-117082, which leads inhibition of metastasis. High FOXC1 expression levels in melanoma cells show more sensitivity to the mTOR inhibitor, rapamycin, which increases apoptosis in cancer cells. However, FOXC1 contributes to the resistance of melanoma cells to PLX4032 (a BRAF inhibitor). Conversely, enhanced FOXC1 expression in breast cancer results in resistance to the DNA-damaging chemotherapy agent FEC. FOXC1 overexpression can also induce resistance to anthracycline-based adjuvant chemotherapy in breast cancer. In addition, resistance to anti-Hedgehog drug, GDC-0449, induced by FOXC1 is also observed in BLBC cells. FOXC1 inhibits GATA3-ERα signaling to exhibit resistance to estradiol and tamoxifen treatment. In addition, FOXC1 significantly decreased cellular responses to docetaxel, which can repress Bcl-2 to increase cell apoptosis. The drug response of FOXC1 is represented by the red dotted lines, and the drug resistance of FOXC1 is indicated by the gray dotted lines. The effects of the drugs are highlighted in blue.
Bcl-2, B-cell lymphoma 2; BRAF, v-Raf murine sarcoma viral oncogene homolog B; ERα, estrogen receptor α; FOXC1, forkhead box C1; mTOR, mechanistic target of rapamycin; NF-κB, nuclear factor kappa light chain enhancer of activated B cells; PARP, poly ADP-ribose polymerase.

migration, and invasion. Furthermore, since FOXC1 may act as a read-out of EGFR-NF-κB activity, FOXC1 can significantly serve as a predictive marker for selecting patients who may benefit from anti-EGFR therapy.[68] High expression of FOXC1 also changed the sensitivity to drugs in melanoma.[36] Melanoma M219 cells that overexpressed FOXC1 exhibited more sensitive to rapamycin (an mTOR inhibitor) compared with M129 control cells. Inversely, M219 FOXC1 cells are more resistant to PLX4032 (a BRAF inhibitor).[36]

FOXC1 and drug resistance

Elevated FOXC1 expression has been reported to transcriptionally attenuate the expression of estrogen receptor α (ERα) through competition with GATA3, an essential regulator of ERα, for binding at the promoter region of ERα.[106] Importantly, this inhibitory effect on ERα induced by FOXC1 reduces cellular responses to estradiol (E2) and tamoxifen in MCF-7 and T47D breast cancer cells, whereas the resistance can be reversed by FOXC1 knockdown in BT549 and HCC1806 cells.[106,107] FOXC1 can also act as a mediator of drug resistance to breast cancer conventional therapy, such as 5-fluorouracil/epirubicin/cyclophosphamide (FEC) and docetaxel.[48] Increased FOXC1 expression in MDA-MB-468 cell lines results in an overt resistance to FEC, a DNA-damaging chemotherapy. However, decreased FOXC1 expression induced by FOXC1 shRNA in MDA-MB-231 cells significantly augmented sensitivity to docetaxel treatment. Moreover, Han and colleagues[81] also reported resistance to anti-Hedgehog (Hh) drugs induced by FOXC1 in BLBC cells. In different BLBC cell lines, elevated expression of FOXC1 can increase the viability of

Table 3. The prognostic significance of FOXC1 in tumor cases.

Tumor type	Research case	Method	Correlation between the FOXC1 expression level with tumor prognosis	Reference
BLBC	2,073 759	cDNA microarray Tissue microarray IHC	Elevated FOXC1 expression increases distant metastasis but decreases metastasis-free survival.	Ray *et al.*[8] Ray *et al.*[16]
HCC	406	Tissue microarray IHC	Positive FOXC1 expression in patients is correlated with shorter OS and higher recurrence rates.	Xia *et al.*[17]
GC	120	IHC	Patients with high FOXC1 expression have remarkably poor recurrence-free and OS.	Xu *et al.*[25]
NSCLC	125	IHC	Patients with upregulated FOXC1 have a significantly shorter disease-free survival duration and OS duration.	Wei *et al.*[18]
ESCC	82	qRT-PCR	Overexpression of FOXC1 in patients correlates with worse prognosis and lower survival rate.	Pan *et al.*[26]
PDA	85	IHC	Increased FOXC1 is inversely associated with patients' vital status and 5-year overall survival rate.	Wang *et al.*[21]
AML	244	Tissue microarray IHC	FOXC1 overexpression is associated with decreased OS and event-free survival, as well as increased disease relapse and refractoriness to induction chemotherapy.	Swaminathan *et al.*[72]
Melanoma	336	Tissue microarray IHC	Lower distant metastasis free survival rate among patients with high FOXC1 expression.	Wang *et al.*[36]
Cervical carcinoma	219	IHC	Patients in high FOXC1 expression group have obviously worse OS and shorter time to recurrence.	Huang *et al.*[23]
Serous ovarian tumor	80	IHC	High expression of FOXC1 serves as a marker for benign serous ovarian tumors and is associated with increased survival rate.	Wang *et al.*[19]

AML, acute myeloid leukemia; BLBC, basal-like breast cancer; ESCC, esophageal squamous cell carcinoma; FOXC1, forkhead box C1; GC, gastric cancer; HCC, hepatocellular carcinoma; IHC, immunological histological chemistry; NSCLC, non-small cell lung cancer; OS, overall survival; PDA, pancreatic ductal adenocarcinoma; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction.

cancer cells with reduced sensitivity to GDC-0449, an SMO-targeting inhibitor, which has been approved by US Food and Drug Administration (FDA) for the treatment of basal cell carcinoma. However, when FOXC1 is repressed by siRNAs in these cells, the acquired GDC-0449 resistance is correspondingly decreased. Furthermore, a mouse xenograft model was established by orthotopic injection of MDA-MB-468 cells into mammary glands and revealed that overexpression of FOXC1 can also abolish the inhibitory effect of GDC-0449 *in vivo*.[81] A recent case–control study has also shown that overexpressed FOXC1 can induce resistance to anthracycline-based adjuvant chemotherapy in sporadic triple-negative breast cancer.[108]

Collectively, the contradictory effects of FOXC1 in cancer therapy may confer more challenges as well as more options for patient management. Although recently published studies have demonstrated the critical function of FOXC1 in cancer diagnosis and treatment,[6,109] the mechanisms underlying these divergent phenomena still need to be illuminated to improve clinical cancer therapy.

FOXC1 serves as a prognostic clinical biomarker in tumors

Over the past 10 years, FOXC1 overexpression has been regarded as an independent predictor of recurrence and survival after curative resection of various human cancers and corresponds to a favorable or unfavorable prognostic significance according to the type of cancer (Table 3).

In BLBC, Ray and colleagues[8] found that high FOXC1 expression levels are negatively correlated with overall survival (OS) based on approximately 2073 total breast cancer patient samples. In the case of lymph-node-negative patients, the

incidence of brain and lung metastasis is increased, and metastasis-free survival is significantly decreased with elevated FOXC1 mRNA expression.[8] Furthermore, the same research group detected FOXC1 protein levels in 759 patients with primary infiltrating invasive ductal breast cancer and found that FOXC1 protein expression detected *via* IHC in triple-negative phenotype (TNP) primary breast cancer is an essential independent prognostic marker that is superior to IHC surrogates of the basal-like subtype.[16] In addition, compared with basal cytokeratins 5/6 (CK5/6), a potent molecular marker for basal-like subtypes of breast cancer, FOXC1 might serve as a kind of functional protein and a candidate for treating BLBC.[16] Furthermore, increased FOXC1 expression was also identified to be positively associated with brain ($p = 0.04$) and lung ($p = 0.01$) metastasis by Jensen and colleagues, which may suggest worse outcome of BLBC patients.[15] Of note, they conducted a FOXC1-based two-tier assay [IHC ± quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)] to rapidly and accurately detect FOXC1 expression in breast cancers.[15] Importantly, the knowledge of FOXC1 cannot be utilized to conduct treatment decisions in the clinic until the emergence of a simple and accurate clinical-grade assay to detect its expression. Therefore, such a commercially available clinical-grade assay significantly promises to improve the diagnostic and prognostic value of FOXC1.

To evaluate the prognostic value of FOXC1, Xia and colleagues[17] confirmed the overexpression of FOXC1 in a tissue microarray of 406 paired HCC samples. Cox's multivariate proportional hazards model revealed that increased FOXC1 expression is related with a worse disease-specific survival for HCC patients and is an independent unfavorable prognostic factor after curative resection. In addition, the negative correlation between upregulated FOXC1 with reduced disease-free survival and OS duration have been analogously observed in GC,[25] pancreatic ductal adenocarcinoma (PDA),[21] NSCLC,[18] ESCC,[26] AML,[72] melanoma,[36] and cervical carcinoma.[23]

Conversely, there is another evidence indicating that overexpression of FOXC1 exists as an advantageous prognosis factor in serous ovarian tumor. Wang and colleagues[19] detected the expression levels of FOXC1 in histological differentiation in 80 serous ovarian tumor tissue samples *via* IHC and identified that augmented expression of FOXC1 was positively associated with well-differentiated benign tumors. Thus, overexpression of FOXC1 may serve as a marker for benign serous ovarian tumor and good prognosis.

Conclusions and perspectives

Over the past 20 years, transcription factors have become a popular area of research in tumorigenesis. FOXC1, a newcomer to the FOX family, has been demonstrated to be upregulated in a wide variety of human cancers as an essential regulator that functions in numerous aspects of tumor progression.[109] Considering the inconsistent effects of FOXC1 in controlling cell proliferation, the traditional terms 'oncogene' or 'tumor suppressor' may be not suitable for FOXC1. However, most basic studies strongly support its pivotal role in conferring aggressive cancer cell traits such as stem-cell-like properties and metastatic propensity. To better understand the oncogenic or oncostatic role of FOXC1, further studies should focus on: (i) whether upregulation or disparate intracellular location of FOXC1 is a cause or a consequence of the progression from normal tissue to carcinoma; (ii) which type(s) of post-transcriptional or post-translational regulation contributes to its upregulation or activation in tumorigenesis; and (iii) the identification of more downstream targets or pathways activated by FOXC1 in cell apoptosis and their connections in tumorigenesis.

Moreover, studies regarding the role of FOXC1 in drug response and drug resistance also reinforce the notion that the role of FOXC1 in cancer is ambiguous. Considering that most data were obtained using cell lines or murine models, future investigations might be implemented with primary tumor cells to elucidate these mechanisms. Another important issue is that the discordant effects of FOXC1 in cell proliferation may partly originate from the underlying differences in cellular context that are cell-type specific and need to be clarified by a more unified study design.

From a therapeutic perspective, downregulating FOXC1 in most human cancers has emerged as an attractive target in many recent studies. Misra and colleagues[110] successfully assembled novel carotenoid functionalized dendritic nanoparticles (CDN) for small interfering RNA of FOXC1 (siFOXC1) delivery in HepG2 cells. Carotenoids are in the purlieu of constituent molecules of biological membranes such as lipids in organized structures and can be assimilated into this complex system at

the correct position and orientation. The CDN-siFOXC1 complex improves the efficiency of *FOXC1* gene knockdown compared with commercial formulations and treatments with particles that have no carotenoid tagging.[110,111] In addition, this CDN-siFOXC1 complex also displays a significantly reduced toxicity profile compared to standard siRNA transfection agents. Thus, these findings provide a novel therapeutic option to efficiently knock down FOXC1 and support the clinical translation of this approach.

Epigenetic alterations of FOXC1 gene can efficiently silence its expression, which may be helpful for cancer therapy. *FOXC1* methylation has been shown as a protective factor against tumor invasiveness in human breast cancer.[112] In 2010, De jeux and colleagues[113] identified a significant difference in survival between methylated and unmethylated samples in human breast cancer, that is, patients with FOXC1 methylated at the promoter region had better survival. In addition, doxorubicin treatment-enhanced methylation of the FOXC1 promoter and consequently improved the OS of patients. In 2011, Kuhmann and colleagues[114] found significant hypomethylation of FOXC1 in MCF7 breast cancer cells after treatment with fractioned ionizing radiation (FIR; 5 times a week with IR in fractions of 2 Gy, resulting in total doses of 10 and 20 Gy), which ultimately leads to radiation resistance in cancer cells. Thus, the resistance to radiation treatment induced by decreased *FOXC1* methylation may be a crucial factor that causes radiotherapeutic failure. However, further insight into the target genes activated by *FOXC1* demethylation that favor cell survival and proliferation in resistance to chemotherapy and radiotherapy could improve the rates of therapeutic failure in clinic. Furthermore, it is necessary to illustrate whether the protective effects of *FOXC1* methylation can be observed in other cancer types.

Furthermore, detection of FOXC1 expression can be used to indicate prognosis and provide valuable information for improved treatment stratification and personalized therapeutic regimens.[109] Therefore, future clinical investigations may concentrate on: (i) defining the standards for assessing FOXC1 concentration to predict clinical outcomes; (ii) determining how to maintain FOXC1 at appropriate levels to enhance therapeutic response and prevent cancer relapse; and (iii) confirming whether the clinical application of therapeutics targeting FOXC1 has other unsatisfactory side effects. Altogether, with the development of transcriptomics, proteomics, and drug screening technologies, further investigations regarding FOXC1 and its oncogenic effects will facilitate improved applications of FOXC1 in cancer treatment.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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