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FOXC1: an emerging marker and therapeutic target for cancer

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Abstract

The Forkhead box C1 (FOXC1) transcription factor is involved in normal embryonic development and regulates the development and function of many organs. Most recently, a large body of literature has shown that FOXC1 plays a critical role in tumor development and metastasis. Clinical studies have demonstrated that elevated FOXC1 expression is associated with poor prognosis in many cancer subtypes, such as basal-like breast cancer (BLBC). FOXC1 is highly and specifically expressed in BLBC as opposed to other breast cancer subtypes. Its functions in breast cancer have been extensively explored. This review will summarize current knowledge on the function and regulation of FOXC1 in tumor development and progression with a focus on BLBC as well as the implications of these new findings in cancer diagnosis and treatment.

Introduction

Forkhead box (FOX) family members are a group of transcription factors characterized by an approximately 100-amino acid winged-helix or forkhead DNA-binding domain.[1] FOX family proteins are involved in many biological processes, including cell proliferation, differentiation, survival, and death (reviewed in refs[2, 3]). In addition to its role in physiological processes, deregulation of FOX proteins is also involved in the development and progression of tumors,[3, 4] raising the possibility that FOX proteins could be used as diagnostic markers and therapeutic targets for cancer.

As a member of the FOX family, FOXC1 has its own unique structure and function. The FOXC1 gene is mapped to chromosome 6p25.[5] Structurally, FOXC1 consists of a N-terminal transactivation domain, forkhead DNA-binding domain, transcription-inhibitory domain, and C-terminal transactivation domain (Figure 1).[6,7] The forkhead domain has a consensus DNA-binding site sequence GTAAATAAA.[8] Compared with other members of

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CONFLICT OF INTEREST

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the FOX family, FOXC1 has critical functions in both physiological and pathological conditions (Figure 2). Classically, FOXC1 plays an important role in the different stages throughout embryonic development. Accumulating evidence has demonstrated its role in tumor development and progression, including breast cancer, acute myeloid leukemia (AML), hepatocellular carcinoma, and many other cancer subtypes. FOXC1 mutations are rarely seen in cancer, whereas its mutations are implicated with Axenfeld–Rieger (AR) syndrome,[5, 9, 10] an autosomal dominant disease that primarily affects the development of eye and bone.[11] In this review, we will summarize the functions of FOXC1 in development and cancer biology, especially its role in basal-like breast cancer (BLBC), the most aggressive breast cancer subtype.

FOXC1 in embryonic development

FOXC1 mRNA is detected in multiple embryonic tissues, including the paraxial mesoderm at the early gastrulation stage, presomitic mesoderm at 8.5 days post coitum, periocular mesenchyme, cornea, mesonephric tubules and surrounding mesenchyme, and endothelial cells.[12,13] Those extensive expression patterns indicate the necessity of FOXC1 in early embryonic development. Indeed, FOXC1-knockout mice die at birth with many defects including hydrocephalus, eye defects, and multiple skeletal abnormalities.[13] These defects might be associated with the function of FOXC1 in the differentiation of prechondrogenic mesenchyme and meningeal cells.[13] Consistent with these results, further study showed that the FOXC1-regulated meninges is involved in cortical development.[14,15] Moreover, FOXC1 is required for cerebellar growth and development,[16,17] reinforcing its role in embryonic neural crest specifications.[18] The involvement of FOXC1 in bone has also been explored mechanistically. For example, the development of mouse calvarial bone requires the bone morphogenetic protein (BMP)-induced Msh homeobox 2 (MSX2) and homeobox protein aristaless-like 4 (ALX4) expression, both regulated by FOXC1.[19] Further study showed that FOXC1 can bind directly to the *MSX2* promoter and upregulate gene expression to induce osteoblast differentiation.[20] Interestingly, FOXC1 could also negatively regulate MSX2 expression by reducing the responsiveness of *MSX2* to BMP-induced SMAD1/5.[21] Thus, FOXC1 regulation of *MSX2* is dependent on its cellular context. FOXC1 is also indispensable for endochondral ossification by interacting with the hedgehog signaling transcription factor GLI2,[22] a similar mechanism also found in the regulation of cancer stem cell (CSC) properties in BLBC.[23]

FOXC1 together with FOXC2, another member of the FOX family, control developmental processes, such as somitogenesis. In the compound *FOXC1/FOXC2* mutant homozygote mice, these animals have a complete absence of segmented paraxial mesoderm, including anterior somites.[24] Deletion of FOXC1 and FOXC2 specifically in paired box gene 3 (PAX3)-positive cells affects cell fate determination in the dermomyotome of somites at the forelimb level, promoting the myogenic cell fate at the expense of endothelial cells that migrate to the limb.[25] Similarly, in zebrafish, FOXC1 inhibition blocked the formation of morphological somites.[26] Both FOXC1 and FOXC2 are also required for the development of the kidney and urinary tract.[27] Another study showed that FOXC1 and FOXC2 regulate the establishment of paraxial versus intermediate mesoderm cell fates in both mouse and chicken embryos.[28]

FOXC1 also plays a role in reproductive development during embryogenesis. The ovaries in FOXC1-knockout embryos are smaller than wild-type controls and contain fewer germ cells.[29] This is due to a majority of the germ cells not migrating to the gonadal ridge in FOXC1-knockout embryos, even migrated cells fail to develop into mature follicles.[29] Although the underlying mechanism still remains unclear, it has been well-established that FOXC1 plays a critical role in cancer cell migration. The molecular basis of FOXC1 function in migration might be shared by both physiological and pathological conditions.

FOXC1 in basal-like breast cancer (BLBC)

Besides its role in embryonic development, accumulating evidence demonstrates that FOXC1 plays critical roles in cancer progression. Elevated FOXC1 expression is associated with poor prognosis in various cancer types, in particular BLBC. It is involved in multiple steps of tumor progression, including cancer cell migration and invasion (Table 1).

FOXC1 as a biomarker for BLBC

Breast cancer is a highly heterogeneous disease with respect to both clinical and molecular features. Based upon gene expression profiles, breast cancer has been classified into four major subtypes, including luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-enriched, and basal-like.[30] Gene expression analysis of human breast cancer microarray data sets showed that compared with other molecular subtypes, FOXC1 is specifically and exclusively expressed in BLBC.[31,32]

Traditionally, BLBC was identified by the triple-negative phenotype (estrogen receptor-negative (ER−), progesterone receptor-negative (PR-), and HER2-negative (HER2-)), plus immunohistochemistry (IHC) staining of the epidermal growth factor receptor (EGFR) and cytokeratins 5/6 (CK5/6).[33] However, a retrospective IHC study showed that triple-negative plus FOXC1 staining showed a superior prognostic relevance in BLBC than compared to triple-negative alone or triple-negative plus basal CK staining.[34] Consistent with this notion, performing a FOXC1-based IHC assay (+/− quantitative real-time polymerase chain reaction (qRT-PCR)) demonstrated that FOXC1 expression is a reliable diagnostic biomarker for BLBC.[35] The PAM50 test, which measures RNA expression profiles of 50 genes to determine a risk of recurrence,[36] has been commonly employed to classify the intrinsic subtypes of breast cancer samples. Notably, compared with this multi-gene signature assay, the FOXC1-based IHC test demonstrated a comparable specificity and sensitivity to identify BLBC,[35] suggesting that the FOXC1-based IHC assay may be established as a rapid, accurate, and cost-effective method for the clinical diagnosis and prognostication of BLBC.

Clinical outcomes associated with elevated FOXC1 expression in BLBC

Analysis of different human breast cancer cDNA microarray datasets showed that elevated FOXC1 expression is associated with a worse overall survival,[31,32] which is consistent with the results from a retrospective immunohistochemistry study of archived breast tumor tissue.[34] Multivariate analysis further showed that the prognostic ability of FOXC1 in predicting breast cancer clinical outcome was independent of other common clinicopathologic factors such as age, tumor size, and lymph node status.[31] Moreover,

FOXC1 expression is positively associated with brain metastasis and significantly with shorter brain metastasis-free survival in breast cancer.[31] Likewise, FOXC1 expression positively correlates with breast cancer lung metastasis.[35] These data suggest a potential regulatory role of FOXC1 in brain and lung metastasis of breast cancer, further establishing FOXC1 as a marker for BLBC which is known to possess a propensity for spreading to the lung and brain. Of note, FOXC1 expression also predicts poor prognosis in many other cancer types (refer to Table 1 and later sections)

Functions of FOXC1 in BLBC

The unambiguous association of FOXC1 levels with breast cancer clinical outcome can be explained, at least in part, by the reported effect of FOXC1 on BLBC cell functions (Figure 3). Providing a mechanism for the long-held notion that BLBC harbors high nuclear factor-κB (NF-κB) activity, Wang et al. showed that FOXC1 can activate NF-κB signaling by increasing p65/RelA protein stability through up-regulating peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 expression in BLBC cells.[37] This FOXC1-activated NF-κB signaling pathway may mediate the FOXC1-induced proliferation, migration, and invasion of BLBC cells.[37] Another study showed that the invasion-promoting function of FOXC1 may also depend on the up-regulation of matrix metalloproteinase-7 (MMP7) expression,[38] which was supported by the finding that knockdown of MMP7 attenuated FOXC1-induced invasiveness of the immortalized human mammary epithelial cell line MCF10A.

Epithelial-to-mesenchymal transition (EMT) is a process performed by embryonic cells to migrate to form tissues and organs.[39] It has been demonstrated that EMT is also involved in cancer metastasis.[40] Recent studies have shown that FOXC1 induces EMT in breast cancer cells. Overexpression of FOXC1 in MCF12A mammary epithelial cells induces EMT, as determined by decreased E-cadherin and increased vimentin expression.[41] Among breast cancer patient samples, FOXC1 is one of the top EMT-inducer genes.[32] Interestingly, circulating tumor cells (CTCs) from breast cancer patients, which undergo EMT and are associated with distant metastasis, express high levels of FOXC1.[42,43] It is worth noting that even though FOXC1 induces metastasis, the role of EMT may have broader implications. Two recently published studies using an *in vivo* lineage tracing mouse model showed that EMT is not necessary for metastasis, but instead induces chemoresistance.[44,45] Consistent with this observation, FOXC1 levels has been shown to be associated with chemoresistance in breast cancer cell models and hypermethylation of the FOXC1 promoter, suggesting that suppression of FOXC1 expression may predict improved survival in breast cancer patients treated with chemotherapy.[46,47]

In addition to the functions mentioned above, FOXC1 is also involved in other functions in breast cancer development. Overexpression of FOXC1 in BLBC cells increases breast CSC properties, which are characterized by CD24-CD44+, ALDH+, CD133+, and mammosphere formation.[23] Further study showed that the increase of CSC properties is mediated by the FOXC1-activated smoothened (SMO)-independent hedgehog signaling pathway,[23] further implicating FOXC1 as a potential therapeutic target for BLBC treatment. Much effort has gone into the understanding of the biology behind that BLBC is poorly differentiated with a mammary progenitor phenotype and is an aggressive breast cancer subtype.[48,49] Because

accumulating evidence indicates that CSC contribute to tumor progression,[50] FOXC1-induced CSC properties in BLBC may explain, at least in part, the aggressiveness and short overall survival of the disease.

During mammary gland development and breast cancer progression, ER plays an essential role and its expression is precisely regulated.[51] BLBC usually lacks the expression of ER, however the underlying mechanism is poorly understood. A previous study showed that the binding of GATA binding protein 3 (GATA3) to the promoter of *ESR1* is critical for the induction of ER expression in breast cancer cells.[52] Our recent study found that FOXC1 competes with GATA3 for the same binding regions in the cis-regulatory elements upstream of the ER gene and thereby down regulates ER expression and consequently its transcriptional activity,[53] providing a possible mechanism underlying the loss of ER expression in BLBC cells. Clinically, loss of ER expression in recurrent breast tumors is a prevalent phenomenon.[54,55] According to our study, a large percentage of breast cancer patients with ER+ primary tumors who received adjuvant tamoxifen treatment recurred as ER−.[53] Not surprisingly, FOXC1 expression was associated with decreased or undetectable ER expression in recurrent tumors,[53] reinforcing its involvement in the repression of ER expression.

Notably, different subtypes of breast cancer exhibit preferential organ-specific distant metastasis. BLBC tends to metastasize to the lung and brain but rarely to bone, comparatively.[56] FOXC1 overexpression is shown to be associated with a shorter brain metastasis-free survival and a better bone metastasis-free survival.[31,35] However, the underlying molecular mechanisms are as yet unknown. A recently published paper showed that the tumor cells disseminated to brain lose phosphatase and tensin homolog (PTEN) expression, which activates NF-κB signaling and then induces C-C motif chemokine ligand 2 (CCL2)-dependent recruitment of myeloid cells to promote tumor outgrowth in the brain.[57] As we mentioned above, FOXC1 activates NF-κB signaling in BLBC cells,[37] thus, FOXC1 might contribute to the establishment of a favorable microenvironment for the tumor cells disseminated to the brain. The FOXC1-induced EMT and migration can also promote brain metastasis through facilitating the penetration of tumor cells through the blood-brain barrier. BMP signaling is known to be an important regulator of bone cell function and bone formation. In light of a recent report showing that FOXC1 activates BMP signaling to impose cell cycle quiescence in hair follicle stem cells (HFSC),[58] one can speculate that the bone microenvironment or the bone marrow niche may induce FOXC1+ breast cancer cells to enter cell cycle arrest. This is supported by the report showing the presence of ER− and BLBC cells in the bone marrow of a large percentage of patients with breast cancer.[59] The possible functions of FOXC1 in brain and bone metastasis need to be tested in the future work.

Regulation of FOXC1 in BLBC

Despite the extensive functional studies in embryonic development and tumor progression, how FOXC1 is regulated is still not well understood, especially in cancer (Figure 4). The reported underlying mechanisms involve both biochemical and epigenetic pathways. In EGFR+ BLBC cells, FOXC1 can be upregulated by EGF in a dose- and time-dependent

manner.[60] Of note, EGFR has been used as one of the surrogate IHC markers to define BLBC.[33] EGFR-induced RAS/extracellular-regulated kinase (ERK) and phosphatidylinositol 3-kinase(PI3K)/AKT signaling pathways promote FOXC1 expression.[60] In agreement, the expression of FOXC1 and EGFR was found to be positively correlated in human breast tumors.[60] EGF can also regulate FOXC1 transcriptional activity through controlling its protein stability.[6] Serine-272, a residue localized in the inhibitory domain of the FOXC1 protein[7] (see Figure 1), can be phosphorylated by EGF-activated ERK signaling, resulting in stabilization of the FOXC1 protein.[6] Coinciding with this post-translational regulation, FOXC1 transcriptional activity can also be controlled by its intrinsic protein conformation, which depends on modification of its transcriptional activation and inhibitory domains.[7] Another regulatory mechanism of FOXC1 expression in BLBC cells was demonstrated in a recent finding that breast cancer susceptibility gene 1 (BRCA1) and GATA3 can interact with each other at the *FOXC1* promoter to co-repress FOXC1 transcription.[46] Interestingly, this study provides a likely explanation for the intrinsic gene expression pattern of FOXC1 in distinct breast cancer subtypes. As mentioned above, FOXC1 is only highly expressed in BLBC, but not other subtypes.[31] Given the fact that the expression of GATA3 is frequently observed in ER+ breast tumor cells, but not BLBC cells,[52] and that BRCA1 mutations are highly prevalent in BLBC,[61,62] it is reasonable to propose that the lack of GATA3 expression together with the presence of low or mutant BRCA1 may reverse FOXC1 repression, leading to BLBC-specific expression of FOXC1.

The expression of FOXC1 in breast cancer can also be regulated by DNA methylation of its promoter region. One report showed that *FOXC1* methylation status was different among pre-treated, locally advanced breast tumors, indicating that increased *FOXC1* promoter methylation might be a protective factor against tumor invasiveness during neoadjuvant doxorubicin treatment.[47] However, *FOXC1* methylation status in other reports seem to contradict the tumor-promotion function of FOXC1. For example, compared with the early stage of invasive breast cancer, *FOXC1* methylation status increases significantly in late-stage disease.[63,64] In agreement with this finding, compared with primary breast tumors, the *FOXC1* methylation level is much higher in matched distant metastasis.[41] This perplexing observation may be explained by the intrinsic expression pattern of FOXC1 in different breast cancer subtypes, in which FOXC1 is highly expressed in ER− but not ER+ breast tumors. Indeed, in the aforementioned analysis, the DNA methylation level of *FOXC1* in ER + breast tumors were substantially higher than ER− tumors.[63,64] In the matched primary and metastatic tumor study, almost all of the analyzed samples were ER+.[41] In MCF-7 luminal breast cancer cells, which are known to possess undetectable FOXC1 expression, the *FOXC1* promoter is hypermethylated.[65] Interestingly, in the regrown cells which were recovered from the growth arrest induced by ionizing radiation, the methylation level of the *FOXC1* promoter was significantly decreased,[65] suggesting that FOXC1 may be involved in radiotherapy resistance. The regulation of FOXC1 by methylation has also occurred in normal tissues, where CD44+ progenitor-like subpopulation of human mammary epithelial cells have a hypomethylated *FOXC1* promoter and high FOXC1 expression.[41] These studies revealed some upstream regulators of FOXC1 expression and activity. However, a more comprehensive understanding of its regulation in BLBC still needs further investigations.

FOXC1 in other cancer types

The crucial role of FOXC1 in tumor progression is not limited to breast cancer, as it has also been found to be involved in many cancer types. For example, elevated expression of FOXC1 is associated with poor prognosis in hepatocellular carcinoma,[66] pancreatic ductal adenocarcinoma,[67] and gastric cancer.[68] Compared with adjacent normal tissues, hepatocellular carcinoma cells express a higher level of FOXC1, which is associated with poor overall survival and high recurrence rates.[66] FOXC1 promotes hepatocellular carcinoma cell invasion and lung metastasis, which might be mediated by its induction of Neural Precursor Cell Expressed Developmentally Down-Regulated Protein 9 (NEDD9)[66] and increased EMT-mediated microvascular invasion.[69] Another study also reported an alternative mechanism to explain how FOXC1 mediates metastasis in hepatocellular carcinoma. FOXC1 upregulates the expression levels of CCL2 and C-X-C motif chemokine receptor 1 (CXCR1) in hepatocellular carcinoma cells by directly binding to their promoters.[70] The upregulated CXCR1 promotes invasion and metastasis of hepatocellular carcinoma cells. Moreover, CCL2 can recruit tumor-associated macrophages (TAM) to further facilitate metastasis.[70]

A recently published paper showed that FOXC1 is overexpressed in at least 20% AML patients.[71] FOXC1 accelerates the onset of AML through blocking monocyte lineage differentiation and enhancing clonogenic potential.[71] FOXC1 has emerged as a central transcription factor in the regulatory network of renal cell carcinoma[72,73] and cholangiocarcinoma.[74] Elevated FOXC1 expression is associated with poor outcome in non-small cell lung cancer patients.[75] In androgen-independent prostate cancer xenografts, FOXC1 is highly expressed and also involved in prostate cancer progression.[76,77] Moreover, it has been shown that FOXC1 is involved in melanoma.[78] However, the precise mechanism by which FOXC1 exerts its functions in these cancers still remains to be elucidated. Collectively, consistent with its functions in breast cancer, FOXC1 promotes cancer cell proliferation, migration, invasion, and distant metastasis. Moreover, elevated FOXC1 expression is usually associated with a poor prognosis in these cancer types.

The regulation of FOXC1 in these cancer types has also been investigated extensively (Figure 4). For example, the expression of FOXC1 could be inhibited by miR-495 in endometrial cells, suppressing FOXC1-induced cell growth and migration.[79] FOXC1 expression could also be regulated by another miRNA in endometrial cells. During the progression of endometrioid endometrial cancer, the expression level of miR-204 is downregulated, which then releases miR-204-suppressed FOXC1 expression to promote FOXC1-induced migration and invasion.[80] In tongue squamous cell carcinoma cells, miR-639 inhibits FOXC1 expression and blocks FOXC1-mediated EMT.[81] Studies also uncovered some other factors that are involved in the regulation of FOXC1 in other cancers. In hepatocellular carcinoma, FOXC1 could be upregulated by interlukin-8 (IL-8), which is mediated by the binding of hypoxia-inducible factor 1-alpha (HIF-1α) to the FOXC1 promoter.[70] FOXC1 is also a target of JUN signaling in diffuse large B-cell lymphoma.[82] Furthermore, transforming growth factor beta (TGF-β) has been reported to positively or negatively regulate FOXC1, depending on the tumor cell background.[83,84]

Implications of normal cell-related FOXC1 function in cancer

It has recently been demonstrated that FOXC1 is a critical regulator for the niche formation of hematopoietic stem progenitor cell (HSPC). CXC chemokine ligand (CXCL) 12-abundant reticular (CAR) cells, which are both adipogenic and osteogenic progenitors, are essential for HSPC maintenance.[85] Compared with osteoblasts, the expression of FOXC1 mRNA levels are significantly higher in CAR cells of both developing and adult bone marrow.[86] The FOXC1 expression in CAR cells upregulates CXCL12 and stem cell factor (SCF) expression and then inhibits CAR cell differentiation into adipocytes, which is not favorable for HSPC maintenance.[86] Similarly, FOXC1 is also required for the maintenance of HFSC quiescence.[58,87] The expression of FOXC1 is induced upon the activation of HFSC[58] when the quiescent stem cells reenter into the cell cycle. FOXC1 induction activates a group of genes, including nuclear factor of activated T-cells 1 (NFATC1) and BMP signaling which are two critical pathways that control stem cell quiescence to promote a quiescent HFSC identity in response to HFSC activation. These observations are consistent with one of the functions of FOXC1 in BLBC, in which FOXC1 increases CSC properties by cell-intrinsic mechanisms.[23]

Many studies suggest that FOXC1 plays an important role in the promotion of angiogenesis. For example, FOXC1 is expressed in endothelium and smooth muscle cells of blood vessels during mouse embryonic development, and compound *FOXC1/FOXC2* null mouse embryos exhibit cardiovascular developmental defects.[24,88] In embryonic vascular development, FOXC1 interacts with Ets translocation variant 2 (ETV2) to regulate endothelial-specific gene expression.[89] Moreover, FOXC1 can interact with vascular endothelial growth factor (VEGF) and NOTCH signaling to regulate expression of arterial-specific genes in endothelial cells.[90] During the period of fetal brain development, FOXC1 is expressed by brain pericytes and is required for pericyte regulation of vascular development.[91] It is also important for the early stage of vascular formation in the telencephalon[92] and essential for maintaining vascular basement membrane integrity in zebrafish.[93] All these findings imply a potential role of FOXC1 in cancer angiogenesis, a hallmark of tumorigenesis and tumor progression,[94] and thus warrant investigation of FOXC1 in tumor angiogenesis in future studies.

Concluding remarks

FOXC1 was initially identified as an important regulator in embryonic development. So far, it has been shown to be involved in many aspects of embryonic development including brain, eye and heart formation. Moreover, FOXC1 plays a critical role in multiple physiological processes, such as the maintenance of the stem cell niche. Recently published studies demonstrated its involvement in cancer, especially in breast cancer, although the precise functions of FOXC1 in cancer progression still need to be extensively addressed. Based on the comprehensive microarray gene expression and IHC analysis, FOXC1 has been proposed as a simple and accurate diagnostic biomarker for BLBC patients.[31,35] Even though there are considerable overlaps between BLBC and triple-negative breast cancer (TNBC), differences exist between them in regards to gene expression profiles and clinical features.[95] Clinically, BLBC is usually defined by TNBC and immune-staining of CK5/6

and EGFR. As an alternative, BLBC could also be diagnosed by microarray-based gene expression profiles, such as PAM50.[36] These methods may not be ideal ways to define BLBC in terms of cost-effectiveness and convenience. Therefore, it is promising that the FOXC1 expression-based identification of BLBC may significantly simplify the procedure of subtype classification and outcome prognostication in patients and may also be useful in guiding therapy selection for BLBC patients. Given the findings that FOXC1 activates SMO-independent hedgehog signaling[23] and mediates EGFR function in basal-like tumors,[60] intervention in the FOXC1 pathway may provide new effective modalities for BLBC treatment. Furthermore, FOXC1 may serve as a predictive biomarker for identifying those patients who may or may not benefit from anti-EGFR, anti-hedgehog, and other targeted therapies.

Although some studies have pursued to elucidate the regulation of FOXC1 in cancer cells, a comprehensive understanding of its regulation network in tumor cells still remains largely unknown. The mechanism underlying the regulation of FOXC1 in BLBC cells could provide insight into how FOXC1 is exclusively expressed in BLBC but excluded from other breast cancer subtypes. The interaction between BRCA1 and GATA3 on the FOXC1 gene promoter provides a possible explanation for the specific expression pattern of FOXC1 among different breast cancer subtypes.[46] However, further investigation is still required to fully understand the signaling networks underlying FOXC1 regulation.

Given the involvement of FOXC1 in embryonic development and breast cancer progression, it is reasonable to rationalize that FOXC1 has a role in mammary gland development. It has been shown that FOXC1 is expressed in mammary luminal progenitor populations.[96] Consistent with this observation, FOXC1 can induce a progenitor-like phenotype in differentiated mammary epithelial cells.[41] Those findings imply a possible link between FOXC1 and mammary gland development. Interestingly, our preliminary data obtained from a FOXC1 transgenic mouse model clearly shows that the mammary gland alveologenesis during pregnancy is completely prevented by FOXC1 overexpression (unpublished data), suggesting a role of FOXC1 in the maintenance of mammary stem/progenitor cell state. However, the detailed mechanism still needs to be further investigated. In agreement with our finding, FOXC1 has a low expression level in the mammary gland during alveologenesis compared to other developmental stages.[97] An interesting concept is that BLBC might be derived from mammary gland luminal progenitor cells.[98,99] Together with its expression in luminal progenitor cells,[96] the possibility of FOXC1 expression in the cellular origin of BLBC is a speculation worth exploring.

In summary, as a critical regulator in embryonic development, FOXC1 has been demonstrated to be involved in tumorigenesis, especially in BLBC. Nevertheless, the detailed mechanism underlying its function and regulation during cancer progression still needs to be clarified. Elucidation of the molecular basis of FOXC1 function would present itself as a potential therapeutic target for FOXC1-overexpressing tumors.

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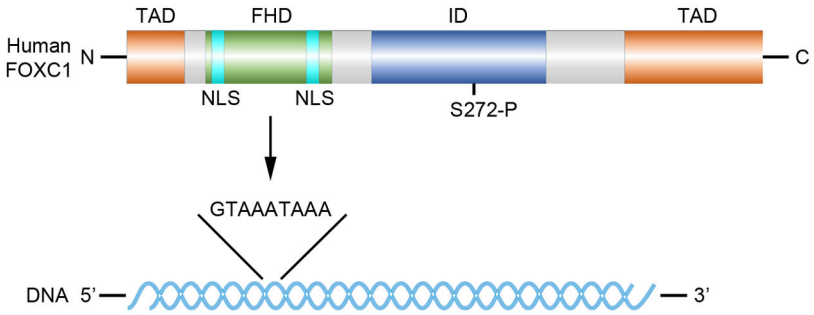

Figure 1. Schematic representation of FOXC1 protein domains and its DNA-binding sequence

Abbreviations: FHD, forkhead domain; ID, inhibitory domain; NLS: nuclear localization sequence; TAD, transactivation domain. Phosphorylation of Ser-272 by EGF-activated ERK signaling is important for stabilization of the FOXC1 protein.

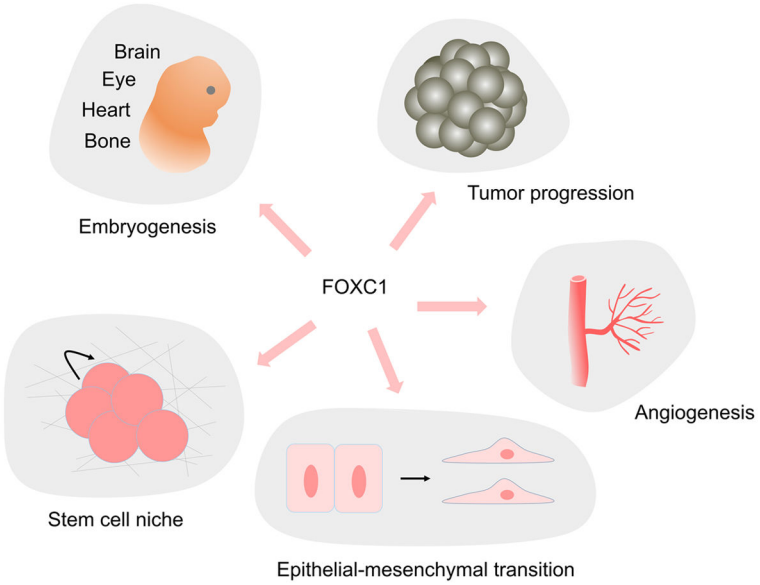

Figure 2. Diagram of FOXC1 functions in physiological and pathological processes

FOXC1 is involved in the development of many organs during embryogenesis, such as brain, eye, heart and bone. FOXC1 exerts its function in many aspects of cancer progression including migration, invasion, and cancer stem cells. FOXC1 also plays crucial roles in physiological processes such as stem cell niche maintenance, epithelial-mesenchymal transition, and angiogenesis.

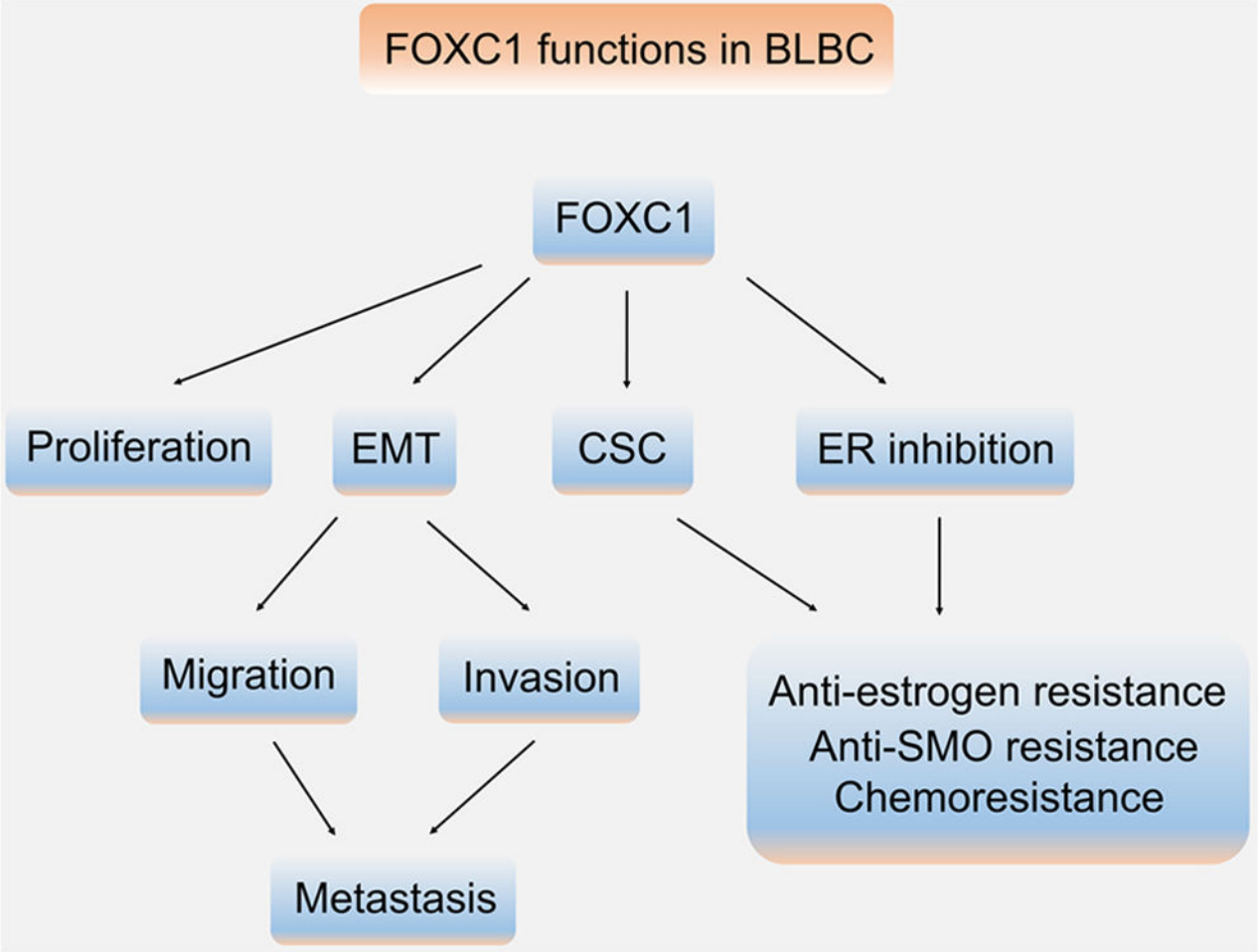

Figure 3. Diagram of FOXC1 functions in BLBC

Abbreviations: BLBC, basal-like breast cancer; CSC, cancer stem cell; EMT, epithelial-mesenchymal transition; ER, estrogen-receptor; SMO, smoothened.

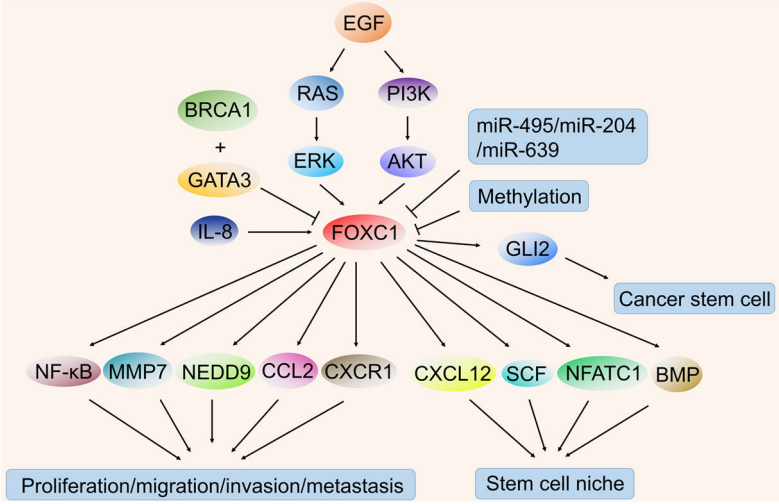

Figure 4.

Schematic illustration of FOXC1-associated signaling pathways in various physiological and pathological conditions.

Table 1

The functions of FOXC1 in different cancer types

Cancer subtypes	Cellular functions	Clinical outcomes	References
Breast cancer	Proliferation, migration, invasion, EMT, metastasis.	Poor prognosis	23, 31, 32, 34, 35, 38, 41
Hepatocellular carcinoma	Invasion, EMT, metastasis.	Poor prognosis	66, 69, 70
Acute myeloid leukemia	Monocyte lineage differentiation block, clonogenic potential.	Unknown	71
Pancreatic ductal adenocarcinoma	Unknown	Poor prognosis	67
Gastric cancer	Unknown	Poor prognosis	68
Renal cell carcinoma	Unknown	Unknown	72, 73
Cholangiocarcinoma	Unknown	Unknown	74
Non-small cell lung cancer	Unknown	Poor prognosis	75
Prostate cancer	Unknown	Unknown	76, 77
Melanoma	Proliferation, migration, invasion.	Poor prognosis	78
Endometrial cancer	Proliferation, migration, invasion.	Unknown	79, 80