FOXQ1 Regulates Epithelial-Mesenchymal Transition in Human Cancers

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Abstract

Epithelial-mesenchymal transition (EMT) in cancer cells plays a pivotal role in determining metastatic prowess, but knowledge of EMT regulation remains incomplete. In this study, we defined a critical functional role for the Forkhead transcription factor FOXQ1 in regulating EMT in breast cancer cells. FOXQ1 expression was correlated with high-grade basal-like breast cancers and was associated with poor clinical outcomes. RNAi-mediated suppression of FOXQ1 expression in highly invasive human breast cancer cells reversed EMT, reduced invasive ability, and alleviated other aggressive cancer phenotypes manifested in 3-dimensional Matrigel (BD Biosciences) culture. Conversely, enforced expression of FOXQ1 in differentiated human mammary epithelial cells (HMLER) or epithelial cancer cell lines provoked an epithelial to mesenchymal morphologic change, gain of stem cell–like properties, and acquisition of resistance to chemotherapeutic-induced apoptosis. Mechanistic investigations revealed that FOXQ1-induced EMT was associated with transcriptional inactivation of the epithelial regulator E-cadherin (CDH1). Our findings define a key role for FOXQ1 in regulating EMT and aggressiveness in human cancer. Cancer Res; 71(8); 1–11. © 2011 AACR.

Introduction

Metastasis of tumor cells to distant organs is the most common cause of death arising from human carcinomas. During the metastatic cascade, carcinoma cells often activate a transdifferentiation step known as epithelial-mesenchymal transition (EMT), a dynamic cellular process thought to underlie metastasis by promoting acquisition of migratory and invasive abilities (1, 2). Evidence is accumulating for a prominent role of EMT in tumor progression (reviewed by Polyak and Weinberg, ref. 3). E-cadherin (encoded by CDH1) has been proposed to be a critical switch in EMT. On downregulation of E-cadherin, epithelial cells acquire a fibroblastic phenotype, dissociate from the epithelium, and migrate. Several proteins have been identified that can downregulate E-cadherin expression including SNAIL, SLUG, ZEB1, ZEB2/SIP1, as well as the basic helix-loop-helix (bHLH) factors TWIST1 and E47 (reviewed by Peinado and colleagues, ref. 4). These proteins bind to E-box elements in the promoter region of E-cadherin, together with other corepressors, leading to transcriptional inactivation of E-cadherin and subsequent breakdown of cell junction and the ensuing loss of cell polarity (5). Consistent with a central role of E-cadherin loss in EMT induction, transcriptional downregulation of E-cadherin is associated with a more aggressive behavior of breast cancer (6).

Cancer cells with different molecular characteristics may have different response to anticancer therapeutics (7). Recently, the induction of EMT in cancer has been associated with increased resistance to chemotherapy. Moreover, the induction of EMT in normal or neoplastic mammary epithelial cell populations has been shown to generate cells with stem-like properties including gain of cell surface antigen CD44/–/CD24/–/low (8, 9), and cancer stem cell–enriched population exhibits increased resistance to chemotherapeutic agents (10–15). These findings have provided an intriguing connection between EMT, cancer stem cells, and drug resistance.

Forkhead transcription factor FOXC2 has been recently implicated in aggressive basal-like breast cancer with a role in regulating EMT and metastasis (16). In this study, we have identified FOXQ1 as another member of the forkhead transcription factor family that is also involved in EMT regulation and is associated with aggressive cancer phenotype. However, unlike FOXC2 that does not seem to affect E-cadherin transcription, FOXQ1-induced EMT is accompanied by transcriptional repression of E-cadherin without engaging other known upstream repressors of E-cadherin.
Materials and Methods

Cell lines and drugs

The cell lines used in this study were purchased from American Type Culture Collection. Immortalized human mammary epithelial cell line HMLE expressing hTERT and SV40 large T and a low level of oncogenic HRAS (Val12) (referred to as HMLER) was a generous gift from Dr. W.C. Hahn at Dana-Farber Cancer Institute. All the basal and luminal breast cancer cell lines are authenticated on the basis of morphology and expressions of epithelial marker E-cadherin or mesenchymal marker vimentin (Vim). HMLER cells of morphology and expression of epithelial marker E-cadherin were cultured in F12 with 5% horse serum, 20 ng/mL EGF, 0.5 mg/mL hydrocortisone, 100 ng/mL cholera toxin, and 10 μg/mL insulin. MDA-MB-231 FOXQ1 knockdown cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% FBS and 2 μg/mL puromycin. All culture reagents and medium were purchased from Invitrogen. 5-Fluorouracil (5-FU), paclitaxel (Taxol), camptothecin (CPT), adriamycin (ADR), and doxycycline (Dox) were purchased from Sigma. Histone deacetylases (HDAC) inhibitor suberoylanilide hydroxamic acid (SAHA) was purchased from Enzo Life Science.

Plasmids and stable cell lines

Full-length FOXQ1 coding region was isolated by reverse transcriptase (RT)-PCR from normal colon tissue. To generate FOXQ1 retroviral expression system, Myc-tagged FOXQ1 was cloned into pMN GFP/ires retrovector (a gift from Dr Linda Penn’s lab), HMLE cells were infected with retrovirus packaged with pMN-FOXQ1-myc and pMN empty vector for 48 hours and followed by GPF sorting. To generate the FOXQ1 Tet-on inducible cell lines, HCT116 stable cell lines expressing tetracyclinecontrolled transcription system were purchased from Addgene. Additional FOXQ1 shRNA sequences was purchased from Santa Cruz, and PLKO-E-cadherin shRNA and PLKO-control plasmid were purchased from Addgene. Additional FOXQ1 shRNA sequence (AGATCAACGAGTACCTCAT) was subcloned into pSIREN-RetroQ-puromycin vector (Clontech), and retroviral particles were produced in Platinum-A cells (Cell Biolabs, Inc.). MDA-MB-231 cells were infected with shFOXQ1 lentiviral and retroviral particles and a null plasmid. Forty-eight hours after transfection, the luciferase activities were analyzed by the Dual Luciferase system according to the manufacturer’s instruction (Promega). Three independent experiments were performed.

Immunofluorescence and immunoblot analysis

Cells were seeded at a density of 10^5 cells/well on 8 mm coverslips in 12-well plates. After 48 hours, coverslips were fixed by ice-cold methanol, and probed with primary Myc-tag (9E10; Roche), E-cadherin (BD610182), or Vim (SC-6260) antibodies in 1/100 to 1/1,000 dilution and florescentlabeled secondary antibodies in a 1/2,000 dilution. Nuclear DNA was stained with 4',6-diamidino-2-phenylindole (DAPI) and coverslips were mounted with FluorSave reagent (CAL-BIOCHEM). Confocal images were taken by Zeiss Meta upright microscope under ×63 oil objective. Immunoblotting was performed as follows: cells were harvested and lysed with radioimmunoprecipitation assay buffer [50 mmol/L Tris-HCl (pH 7.4), 1 mmol/L EDTA, 150 mmol/L NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and protease inhibitors]. Protein concentrations were determined with the Bradford protein assay kit (Bio-Rad). Protein samples (20–30 μg) were separated by SDS-PAGE, transferred onto an Immobilon membrane (Millipore), and blotted with E-cadherin (BD610182), Vim (SC-6260), Myc-tag (9E10, Roche), CD44 (BD550392), CD24 (BD555426), FOXQ1 (Sigma AV9755), and cleaved-PARP (C.S.D214), and actin (Roche).

Cell viability and Caspase-3 activity assay

Cell viability was measured by CellTiter-Glo Luminescent Cell Viability Assay (Promega). MDA-MB-231 cells and HMLER cells were seeded at a density of 1,000 cells/well in 96-well plates. Cells were lysed after indicated days by 100 μL of CellTiter-Glo reagents, and the luminescence signals were measured by MicroLumat Plus LB986 system. Six replicas were performed for each sample. Active Caspase-3 activities were measured by using BD cytofix/cytoperm fixation/permeabilization kit with FITC Rabbit antiactive Caspase-3 antibody (BD559341), based on the manufacturer’s instructions and followed by FACS analysis.

Mammosphere formation assay and 3-dimensional culture on Matrigel

Single-cell suspension of HMLER-FOXQ1 and negative control were plated (5,000 cells/well) in 6-well ultra-low attachment plates (Corning) in MammoCult medium with hydrocortisone and heparin (STEMCELL Technologies) and bottom medium is DMEM with 10% FBS. After 24 hours incubation, inserts were fixed with 3.7% formaldehyde, and stained with propidium iodide. Invaded cells were scanned and counted by using high content screening machine (Cellomics ArrayScan VHT) for 10 individual fields for each insert. At least 3 independent experiments were repeated.
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cultured for 7 days. For mammosphere numbers calculation, single-cell suspension (100 cells/well) were plated in 96-well ultra-low attachment plates in MammoCult medium hydrocortisone and heparin for 7 days, sizes more than 100 μm were counted under microscope. For 3-dimensional (3D) culture of cells on Matrigel, MDA-MB-231 FOXQ1 knockdown and control cells were plated (5,000 cells/well) in 8-well chamber slide (BD) on a solidified layer of 7.6 mg/mL growth factor reduced Matrigel (BD56231) measuring approximately 1 to 2 mm in thickness with DMEM containing 2% FBS and 2% Matrigel and the medium were replaced for every 4 days. Phase contrast images of the 3D structure were taken on day 12.

Microarray analysis and quantitative real-time RT-PCR

Total RNA was isolated by using Trizol (Invitrogen) and purified with the RNeasy Mini Kit (QIAGEN). The microarray hybridization was performed with the Illumina Gene Expression Sentrix BeadChip HumanRef-8 V3, and data analysis was performed by GeneSpring software from Agilent Technologies as described (17). The analyzed gene sets by GeneSpring were next subjected to Ingenuity Pathway Analysis (IPA) for gene ontology (GO) analysis. Quantitative real-time RT-PCR was performed by PRISM 7900 Sequence Detection System (Applied Biosystems). Briefly, reverse transcription was performed by an RNA Amplification kit (Ambion), and cDNA were next amplified by FOXQ1, E-cadherin, Vim, CD44, CD24, and GAPDH Taqman probes (Applied Biosystems). Three independent experiments were performed for quantitative real-time RT-PCR.

Dataset and survival analysis

The breast cancer dataset from the van de Vijver cohort with relevant clinical information has been described previously (18). The expression of probes of each FOXQ1 gene was averaged and transformed to z-score. The positive z-score was treated as higher expression and the negative z-score was treated as lower expression. Kaplan–Meier survival analysis was used for the analysis of clinical outcome. For multivariate analysis, a Cox proportional hazards model was used to estimate the HRs of breast cancer patient survival for FOXQ1, along with estrogen receptor (ER) status (1 for ER + and 0 for ER - ), lymph node status (1 for LN + and 0 for LN - ) and grade (-1 for grade 1, 0 for grade 2, and +1 for grade 3).

Results

FOXQ1 expression is associated with aggressive breast cancer

To identify additional EMT regulators within the forkhead transcription family, we performed gene expression profiling analysis and compared the expression levels of forkhead transcription factor members between the highly invasive and mesenchymal-like breast cancer MDA-MB-231 cells and noninvasive epithelial breast cancer MCF7 cells, by using Illumina Beadchip. The result shows that of all 49 forkhead family members presented in the array FOXQ1 was the most highly overexpressed in MDA-MB-231 cells as compared with MCF-7 cells (Fig. 1A). To further verify the array data and to extend the analysis in other breast cancer cell lines, we further performed quantitative RT-PCR and Western blot analysis in multiple breast cancer cell lines, including aggressive and basal MDA-MB-231, BT549, MDA-MB-468, and MDA-MB-436 cells and nonbasal epithelial MCF-7, T47D, SKBR-3, BT474, MDA-MB-361, and MDA-MB-453 cells, as well as noncancerous mammary epithelial MCF10A and HMLER cells. The results show that FOXQ1 was consistently expressed in higher levels in aggressive breast cancer cells than that in nonaggressive breast cancer cells (Fig. 1B). Of note, FOXQ1 mRNA and protein levels were not always consistent, indicating that FOXQ1 expression might also be subject to post-translational regulation.

To seek clinical evidence linking FOXQ1 expression to breast cancer, we examined the FOXQ1 expression in Oncotype database (19). The data from various breast cancer cohorts showed that the FOXQ1 expression correlated with a higher tumor grade (P = 0.0007), metastasis (P = 0.003), and is highly enriched in unfavorable ER/progesterone (PR)/HER2 triple-negative basal-like subtype (P = 0.008; Fig. 1C). Moreover, higher FOXQ1 expression is also associated with poor clinical outcomes (Fig. 1D, P = 0.002). In a multivariate analysis of FOXQ1 together with ER, lymph node, or tumor grade status, FOXQ1 expression status retained independent prognostic significance (HR = 2.9; P = 0.0078), along with ER- (HR = 0.53; P = 0.01) and high histologic grade (HR = P = 6.3e-5; Supplementary Table S1). These results from both in vitro and clinical data suggest that FOXQ1 might play a role in aggressive subtype of breast cancers.

FOXQ1 depletion reduces mesenchymal phenotype and invasive ability of MDA-MB-231 cells

To functionally validate the role of FOXQ1 in aggressive breast cancers, we established a stable MDA-MB-231 cell line expressing 3 pooled individual short hairpin RNAs purchased from Santa Cruz (shRNA-SC) targeting FOXQ1, and additional 2 stable clones expressing an independent FOXQ1 shRNA (#02-09 and #02-12). Notably, FOXQ1 ablation in all settings induced a change from spindle-like mesenchymal morphology of MDA-MB-231 cells into epithelial morphology by manifesting an increased cell-to-cell adhesion (Fig. 2A). Consistent with the phenotypic change associated with FOXQ1-depletion was an increased expression of epithelial marker E-cadherin (CDH1) concomitant with a downregulation of mesenchymal marker Vim, as determined at both mRNA and protein levels (Fig. 2B). These changes of EMT markers were also verified by immunofluorescent confocal analysis (Fig. 2B). Thus, FOXQ1 depletion resulted in a reversal of EMT in MDA-MB-231 cells. Furthermore, FOXQ1 depletion also markedly reduced the invasive ability of MDA-MB-231 cells in all 3 FOXQ1 ablation cell lines, as assessed by the technique of transwell invasion assay (Fig. 2C). This reduction of invasion should not be due to reduced cell proliferation, as FOXQ1 ablation did not change the proliferation rate of MDA-MB-231 cells (Fig. 2C, bottom). When cultured on Matrigel to assess the 3D growth potential, the control MDA-MB-231 cells displayed aggressive phenotype by showing highly disorganized structures of cell cluster lacking basal polarity, whereas the MDA-MB-231 cells
depleted of FOXQ1 showed more uniform and polarized acinar structures (Fig. 2D).

Enforced expression of FOXQ1 in human mammary epithelial cells induces EMT and mammosphere formation

We next examined whether enforced expression of FOXQ1 in human mammary epithelial cells is able to trigger mesenchymal phenotype. To achieve this, we used a semitransformed human mammary epithelial cell line (HMLER; ref. 20) as a model in which overexpression of EMT regulator Snail or Twist1 has been shown to induce EMT (8). We established a stable HMLER cell line expressing FOXQ1 through retroviral induction (designated as HMLER-FOXQ1). We found that although the HMLER cells expressing a control vector retained an epithelial morphology with tight cell-to-cell adhesion, HMLER-FOXQ1 cells displayed an elongated morphology typically associated with mesenchymal phenotype (Fig. 3A). Further, this morphologic change in HMLER-FOXQ1 cells was accompanied by marked reduction of E-cadherin (CDH1) expression and increased expression of Vim at both mRNA and protein levels (Fig. 3A). These changes of EMT markers were confirmed by immunofluorescent staining of E-cadherin and Vim (Fig. 3A). These findings suggested that enforced expression of FOXQ1 in HMLER cells can result in induction of EMT.

Recent studies have shown that human mammary epithelial cells undergoing EMT often exhibit a gain of stem cell–like characteristics, including changes of certain cell surface markers specifically increased expression of breast stem cell marker CD44, and reduced expression of CD24, as well as enhanced capacity to form spherical colonies in serum-free suspension cultures, termed mammospheres (8, 21). To test whether FOXQ1 overexpression in HMLER cells can also result in similar stem cell–like properties, we assessed the alterations in expression of CD44 and CD24 in
HMLER-FOXQ1 cells as well as the cell’s capacity to form mammospheres. Indeed, HMLER-FOXQ1 cells expressed increased levels of CD44 and reduced CD24 as compared with the control cells (Fig. 3B) in both mRNA and protein levels and formed significantly larger numbers of mammospheres when cultured in suspension by using a mammosphere formation medium for 4 to 7 days (Fig. 3C). The formed mammosphere populations were found to be further enriched in protein levels of FOXQ1 as well as CD44, which supports a role of FOXQ1 in driving the formation of mammosphere (Fig. 3C). Consistent with an established role of E-cadherin loss in EMT induction in mammary epithelial

Figure 2. FOXQ1 depletion reduces EMT and invasive ability of MDA-MB-231 cells. A, cell morphology of MDA-MB-231 control and MDA-MB-231 cells depleted of FOXQ1. B, mRNA and protein levels of FOXQ1, E-cadherin, and Vim from the above cell lines were measured by quantitative RT-PCR (left) and Western blotting (right), respectively. Immunofluorescent staining of E-cadherin and Vim of above cell lines are also shown. C, the invasive ability of above cell lines is presented as total number of cells that have migrated to the bottom chamber as calculated in the sum of 10 fields. Each sample was measured in triplicates, and each experiment was repeated at least 3 times. Left, representative images of cells migrated to the bottom chamber are shown below. Right, the proliferation rate of the 2 cell lines, as measured by CellTiter-Glo viability assay. D, phase contrast images of 3D Matrigel growth of above 2 MDA-MB-231 cells with or without FOXQ1 depletion. Results are typical and representative of 3 independent experiments.
cells (22), we showed that E-cadherin knockdown in HMLER cells was sufficient to induce EMT and mammosphere formation, with corresponding changes in levels of Vim, CD44 and CD24 (Fig. 3D). Thus, E-cadherin loss is a functionally relevant target of FOXQ1 in EMT induction. Collectively, these findings suggested that FOXQ1 is able to induce EMT and stem cell–like characteristics associated with aggressive phenotype of breast cancers.

Figure 3. FOXQ1 induces EMT and stem cell properties in human mammary epithelial cells. A, morphology of HMLER cells expressing control vector (pMN) or FOXQ1 (left); changes of E-cadherin and Vim at both mRNA and protein levels were assessed by quantitative RT-PCR and Western blotting, respectively (middle); immunofluorescent staining of E-cadherin and Vim of HMLER cells expressing control vector (pMN) or FOXQ1 (right). B, mRNA and protein levels of breast cancer stem cell markers (CD44 and CD24) were assessed by quantitative RT-PCR and Western blot analysis. C, top, phase contrast images of mammosphere formation in HMLER cells expressing a control vector (pMN) or FOXQ1. Bottom, left, quantification of numbers of mammospheres from 3 independent experiments (error bar indicates ±SD); right, protein levels of CD44 and FOXQ1 from HMLER-FOXQ1 and corresponding mammosphere populations (FOXQ1-M) were assessed by Western blot analysis. D, phase contrast images of morphology (left, top) and mammosphere formation (left, bottom) of HMLER control and HMLER expressing shEcad. Quantification of mammospheres, and changes of EMT and breast cancer stem cell markers are shown in the middle and right panels, respectively.
Ectopic FOXQ1 expression induces EMT in epithelial colorectal cancer cells

To extend our analysis, we wanted to determine whether FOXQ1 can also induce EMT in epithelial cancer cells. FOXQ1 has been recently found to be overexpressed in colon cancer (23). In a series of colon cancer cell lines that we have examined, FOXQ1 was found to be silenced in HCT116 cells although it is expressed abundantly in other colon cancer cell lines, as determined by both quantitative RT-PCR and Western blot (Fig. 4A). The silencing of FOXQ1 in HCT116 cells was caused by DNA methylation of the promoter as revealed by a large scale DNA methylome analysis in HCT116 cells (data not shown). HCT116 cells exhibit a typical epithelial morphology and thus may provide an ideal model to examine the potential of FOXQ1 to induce EMT in epithelial cancer cells. We established a Tet-on inducible HCT116 cell line, designated as HCT116-FOXQ1, in which FOXQ1 tagged with Myc is expressed on Dox treatment. We found that although the vector control HCT116 cells retained an epithelial morphology with or without adding Dox, HCT116-FOXQ1 cell cultured in the presence of Dox displayed a fibroblast-like morphology (Fig. 4B), reminiscent of EMT induction. Moreover, removal of Dox from the culture medium to eliminate FOXQ1 expression resulted in a reversal of epithelial to mesenchymal morphology (Fig. 4B). Consistent with the acquisition of EMT morphology in HCT116-FOXQ1 cells was the downregulation of E-cadherin and upregulation of Vim (Fig. 4C). Immunofluorescent confocal imaging analysis confirmed the above molecular changes on E-cadherin and Vim at single cell level (Fig. 4D), further substantiating a role of FOXQ1 in inducing EMT in epithelial cancer cells. Therefore, FOXQ1 might have a broader role in modulating cancer EMT. However, we also noticed that ectopic FOXQ1 expression was unable to induce EMT in breast epithelial MCF-7 cells or lung epithelial cancer A549 cells (data not shown). These results suggested that FOXQ1 alone was insufficient, but, depending on the...
cellular context, may require additional oncogenic factors to provoke EMT.

**FOXQ1 overexpression confers resistance to chemotherapy-induced apoptosis**

Cancer cells undergoing EMT has recently been connected to chemoresistance (24). We next investigated whether FOXQ1 has a role to play in drug-induced apoptosis. We found that FOXQ1 depletion in MDA-MB-231 cells resulted in increased apoptotic response to a series of chemotherapeutic reagents, including 5-FU, paclitaxel, and CPT, as assessed by Caspase-3 activation assay (Fig. 5A). Conversely, ectopic expression of FOXQ1 in HCT116 cells resulted in reduced PARP cleavage in response to 5-FU as compared with control HCT116 cells (Fig. 5B). It also decreased apoptotic response to CPT, ADR, or SAHA as assessed by FACS analysis of active Caspase-3 activity (Fig. 5C). Hence, our results showed that FOXQ1 deregulation not only induces EMT to influence cancer progression but can also confer resistance to chemotherapy-induced apoptosis.

**FOXQ1 represses E-cadherin transcription and associated transcriptional program**

We next sought to identify the transcriptional program associated with FOXQ1-mediated EMT. To accomplish this,
we performed transcriptional profiling on HMLER cells expressing either control vector or FOXQ1 as well as MDA-MB-231 cells expressing either control or FOXQ1 shRNA. We aimed at identifying the common gene sets that are either positively or negatively regulated by FOXQ1 in both scenarios. To this end, we have identified a set of 63 genes that were upregulated on FOXQ1 overexpression in HMLER, but downregulated on FOXQ1 knockdown in MDA-MB-231 cells (using 5-fold cutoff; Fig. 6A and B; Supplementary Table S2). Vice versa, a set of 206 genes were found to be downregulated by FOXQ1 (using 5-fold cutoff; Fig. 6A and B; Supplementary Table S3). GO analysis revealed that genes downregulated by FOXQ1 were most enriched for gene sets related to cell morphology or migration and motility (Fig. 6B), thus supporting a functional role of FOXQ1 in regulation of EMT. In contrast, genes positively regulated by FOXQ1 were not enriched with such gene function categories related to EMT (Fig. 6B). In addition, IPA of FOXQ1-downregulated gene set revealed a top gene network with functions in cellular movement, connected with CDH1 (E-cadherin) as a central node (Fig. 6C). These findings suggest that FOXQ1 modulated EMT is able to suppress E-cadherin transcription. However, our chromatin immunoprecipitation (ChIP) analysis failed to detect a convincing FOXQ1 enrichment at the proximal E-cadherin promoter region containing the E-box.

Discussion

Here we have shown a functional role of FOXQ1 in promoting EMT in both human mammary epithelial cells and colorectal epithelial cancer cells. In agreement with in vitro analysis, gene expression in silico analysis of breast cancer gene expression database also revealed that FOXQ1 was expressed at
higher levels in aggressive breast cancer subpopulations and is clinically associated with poorer prognosis of breast cancer patients. Strikingly, FOXQ1 expression is also correlated with most of the colon carcinoma cell lines, and ectopic expression FOXQ1 in one of the FOXQ1-deficient epithelial carcinoma cell not only induce EMT but also make these cells become drug resistant. These results suggest FOXQ1 is envisioned to play a role in the progression of breast cancer in patients, as well as exert functional impact in colon carcinoma. Collectively, FOXQ1 is believed to have a wide influence on tumor development.

Gene expression analysis has shed light on the mechanisms underlying FOXQ1-induced EMT. We found that E-cadherin-associated gene expression program could be a major target inactivated by FOXQ1. Meanwhile, other well-known EMT regulators were not found to be affected by FOXQ1, thereby emphasizing that E-cadherin loss may be the main event in FOXQ1-induced EMT program. Moreover, consistent with previous report (22), we confirmed that E-cadherin knockdown could be sufficient to induce EMT in human mammary epithelial cells. Conversely, a recent paper reported that enforced expression of E-cadherin in mesenchymal-like MDA-MB-231 cells can trigger an epithelial phenotype (25). Thus, transcriptional inactivation of E-cadherin is a functionally relevant target of FOXQ1-induced EMT. Our result therefore distinguishes from another forkhead transcription factor FOXC2, which has been shown to induce EMT without affecting E-cadherin mRNA level, but instead relocal E-cadherin from the plasma membrane to the cytoplasm (9). However, unlike other known E-cadherin repressors, such as SNAIL (26, 27), SIP1 (28), SLUG (29, 30), TWIST1 (31) that downregulate E-cadherin by binding to E-box of the promoter, our ChIP analysis failed to detect an enrichment of FOXQ1 at E-cadherin promoter. Therefore, it remains to be determined which factor transcriptional repress E-cadherin as a direct target during FOXQ1-induced EMT program.

Beginning with the isolation of CD44+/CD24−/low breast tumor-initiating cells (21, 26, 32), a number of studies have isolated tumor-initiating cells or cancer stem cells (CSC) with self-renewal properties in a number of solid malignancies and cancer cell lines (27, 28). Compelling evidence exists relating EMT to the emergence of CSC-like phenotype, which may be prerequisites for cancer cell metastasis (33). Experimental evidence showing a direct connection of EMT to stem cells has recently been reported by Weinberg and colleagues (8). Thus, inducing EMT in differentiated human mammary epithelial cells by repressing E-cadherin gives rise to CD44+/CD24−/low CSCs (7, 8). These phenotype changes were also observed through FOXQ1 overexpression in the same cell system. Moreover, consistent with the high level of expression of FOXQ1 in MDA-MB-231 cells, a recent study has shown that more than 30% of MDA-MB-231 cells display CD44+/CD24−/low phenotype (33). Therefore, these findings may indicate a role of FOXQ1 in cancer EMT and possible CSC regulation.

Cancer therapy has often been associated with acquired resistance. An increasing body of literature now suggests that acquired resistance to chemotherapy is likely to be linked to acquired EMT (34). For instance, elevated E-cadherin expression or increased mesenchymal phenotype can result in resistance to epidermal growth factor receptor kinase inhibitor (27) or DNA damaging agent–induced apoptosis (28). Moreover, chemotherapy leading to an increase in the number of CD44+/CD24−/low CSCs (29) may represent a potentially important mechanism of acquired drug resistance. Therefore, in addition to the gain of self-renewal ability of cancer cells undergoing EMT, they also become chemoresistant at the same time. Consistent with this reasoning, we have shown that FOXQ1 depletion in MDA-MB-231 cells can result in sensitization to a variety of chemotherapeutic agents whereas enforced expression of FOXQ1 in epithelial cancer cells can induce chemoresistance. A recent report indicated that FOXQ1 is overexpressed in colon cancer and contributes to drug resistance by upregulating cell cycle checkpoint regulator p21 (23). Therefore, FOXQ1 may play a broader role with respect to multiple drug resistance in human cancer and this is likely to be achieved through more than one single mechanism.

Here, we emphasize that triple-negative breast cancers (ER/PR/HER2-negative) comprises approximately 15% of total breast cancer population. Despite their high incidence rate, triple-negative breast cancers are extremely difficult to treat and the outcomes are usually not desirable. The other obstacle of breast cancer treatment is their tendency to relapse and metastasize to distant sites. Hence, any treatment regimen that targets proteins involved in EMT pathways and CSC maintenance has to be a promising therapeutic strategy as it aims to eliminate any surviving cancer cells to prevent recurrence while improving the long-term prognosis of breast cancer patients. In view of the relevance of EMT to disease progression in cancer pathology, our work which has shown sensitization of cancer cells to chemotherapy on FOXQ1 depletion therefore opens up a novel prospect for future cancer therapy through modulation of cellular FOXQ1 activities.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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