PDGFRα and β Play Critical Roles in Mediating Foxq1-Driven Breast Cancer Stemness and Chemoresistance

Fanyan Meng1,2, Cecilia L. Speyer3, Bin Zhang4, Yongzhong Zhao4, Wei Chen1,5, David H. Gorski3, Fred R. Miller1,2, and Guojun Wu1,2

Abstract

Many epithelial–mesenchymal transition (EMT)–promoting transcription factors have been implicated in tumorigenesis and metastasis as well as chemoresistance of cancer. However, the underlying mechanisms mediating these processes are unclear. Here, we report that Foxq1, a forkhead box-containing transcription factor and EMT-inducing gene, promotes stemness traits and chemoresistance in mammary epithelial cells. Using an expression profiling assay, we identified Twist1, Zeb2, and PDGFRα and β as Foxq1 downstream targets. We further show that PDGFRα and β can be directly regulated by Foxq1 or indirectly regulated through the Foxq1/Twist1 axis. Knockdown of both PDGFRα and β results in more significant effects on reversing Foxq1-promoted oncogenesis in vitro and in vivo than knockdown of either PDGFRα or β alone. In addition, PDGFRβ is a more potent mediator of Foxq1-promoted stemness traits than PDGFRα. Finally, pharmacologic inhibition or gene silencing of PDGFRs sensitizes mammary epithelial cells to chemotherapeutic agents in vitro and in vivo. These findings collectively implicate PDGFRs as critical mediators of breast cancer oncogenesis and chemoresistance driven by Foxq1, with potential implications for developing novel therapeutic combinations to treat breast cancer.

Cancer Res; 75(3); 584–93. © 2014 AACR.

Introduction

Cancer recurrence, metastasis, and chemoresistance correlate with each other and contribute greatly to the mortality of patients with breast cancer (1–3). For example, metastatic breast tumors tend to be more chemoresistant than primary tumors, as demonstrated by the marked decrease in the chemotherapeutic response rate in the metastatic breast cancer setting versus the neoadjuvant setting (1). In addition, chemoresistant tumors are prone to metastasize and respond poorly to neoadjuvant chemotherapy. This often correlates with earlier metastatic recurrence and shorter disease-free and overall survival (1). However, it remains undetermined whether there is a common mechanistic element linking these processes.

Recently, epithelial–mesenchymal transition (EMT) has been recognized as a mechanism for breast cancer cells to acquire metastatic properties (2, 4–8). Many transcription factors including Snail (9, 10), Twist1 (11), Foxc2 (12), Zeb1, and Zeb2 (13, 14) are capable of triggering EMT, promoting tumorigenesis and metastasis, and enhancing chemoresistance. However, how these transcription factors interact remains elusive, as do the crucial mediators of these EMT-promoting genes. Discovering these mediators could provide insight into the mechanisms of cancer recurrence, metastasis, and chemoresistance, and eventually facilitate the development of a targeted cancer therapy.

Foxq1, a human Forkhead-Box gene family member (15–17), has been shown to repress smooth muscle–specific genes (18) and is involved in hair follicle development (19, 20). Foxq1 is overexpressed in different cancer types (21–23) and known to be induced by TGFβ signaling (24, 25). Consistent with these findings, our laboratory previously reported overexpression of Foxq1 in highly metastatic breast cancer cell lines and a direct correlation between Foxq1 expression and poor outcomes in patients with breast cancer. More importantly, we have also shown that ectopic expression of Foxq1 triggers EMT and contributes to breast cancer metastasis (26), a finding that was confirmed in two follow-up reports (25, 27). In our current study, we demonstrate that Foxq1 contributes to stemness traits and chemoresistance in mammary epithelial cells and that these functions are dependent on the Foxq1/Twist1/PDGFRs transcriptional axis. Using a loss-of-function assay and pharmacologic inhibition, we show that targeting PDGFRs is potentially an effective therapeutic approach to reverse oncogenesis, stemness traits, and chemoresistance driven by Foxq1 in breast cancer.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

The datasets generated in the article have been deposited in GEO under the accession number GSE46834.

Corresponding Author: Guojun Wu, Department of Oncology, Karmanos Cancer Institute, Wayne State University School of Medicine, 4100 John R, HWCRC, Room 840.2, Detroit, MI 48201. Phone: 313-576-8349; Fax: 313-576-8029; E-mail: wuoj@karmanos.org

doi: 10.1158/0008-5472.CAN-13-3029
©2014 American Association for Cancer Research.
Materials and Methods

Cell culture

HMLE cells (human mammary epithelial cells immortalized with SV40 large T antigen and catalytic subunit of telomerase) and HMLER cells (HMLE cells transfected with activated Ras gene) were maintained in the culture as previously described (28). Mouse breast cancer cell line 4T1 was cultured in high-glucose DMEM and supplemented by 10% FBS, NEAA, and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin). NMuMG cells were cultured with DMEM medium with 4.5 g/L glucose, 10 mg/mL insulin and 10% FBS. All cell lines were authenticated upon receipt by comparing them to the original morphologic and growth characteristics.

Generation of gene overexpressing and knockdown stable cells

Full-length plasmids for Foxq1, Twist1, Zeb2, and Snail1 were purchased from Open Biosystems. These genes were then subcloned into a pENTR vector and recombinated into the pLenti-6/Novato lentivirus-expression system (Invitrogen). The generated virus was used to infect targeted model cells. Stable cells were generated after being selected with blasticidin (10 mg/mL, Invivogen; refs. 29, 30).

A set of five shRNA clones for PDGFRα and β were purchased from Open Biosystems. The shRNA sequences for targeting PDGFRα, β and Foxq1 genes were shown in Supplementary Table S1. The lentiviruses for the shRNAs of PDGFRs were generated using the Trans-Lentiviral packaging system (Open Biosystems) for shRNA expression. The generated virus was used to infect targeted model cells. Stable cells were generated after being selected with puromycin (2.5 μg/mL, Invivogen).

Microarray analysis and RT-PCR

RNA was extracted from cells of interest using TRIzol (Invitrogen) and purified using an RNA Purification Kit (Qiagen) according to manufacturer’s instructions. RNA (25 ng) was labeled with dye and applied to the microarray. Changes in gene expression were analyzed using a Sentrix human Ref-8 Expression BeadChip (Illumina, 8 array “stripes”). Data was normalized using the “average” method that simply adjusts the intensities of the two populations of gene expression values such that the means of the populations become equal. Fold enrichment values were used to obtain the list of candidate genes with greater than two-fold change. RT-PCR based on RNA from three independent cell cultures was performed as previously described (31) to validate the microarray result. Primer sequences are shown in Supplementary Table S2.

Cell proliferation, Transwell migration assay, Boyden chamber invasion assay, and clonogenic assay

Detailed methods of these assays are given in the Supplementary Materials and Methods section.

Mammosphere formation assay

A mammosphere formation assay was performed as previously described with the following modifications (32). Briefly, ten thousand cells were plated on a 6-well ultra-low attachment plate (Corning Inc.) and were grown in serum-free mammary epithelial growth medium (MEBM Basal Medium, Lonza) supplemented with B27 (Invitrogen), 20 ng/mL EGF, 1 μg/mL hydrocortisone, 5 μg/mL insulin, and 5 μg/mL β-mercaptoethanol. One milliliter of medium was added every other day for 7 to 12 days. Images of mammospheres were recorded and the number of mammospheres was manually counted. Experiments were performed in triplicate and repeated two times.

Tumor xenograft studies

All mice experiments were carried out in accordance with approved protocols from the Institutional Animal Care and Use Committee at Wayne State University (Detroit, MI). We performed tumor xenograft studies using either BALB/c or NCR nu/nu female mice from NIH to study the effect of Foxq1 in chemoresistance, tumorigenesis and PDGFR’s effect in mediating Foxq1’s function in chemoresistance. The detailed methods are described in the Supplementary Material and Methods.

Statistical analysis

The quantitative results were analyzed using a two-sample t test or one-way ANOVA. If the normality assumption did not hold, these parametric tests were replaced by nonparametric tests Wilcoxon or Kruskal–Wallis tests, respectively. The dose-response curves for doxorubicin or paclitaxel within each cell line type were analyzed using multiple linear regression with interactions and a dummy variable to denote the conditions of imatinib. For analysis of synergism, a 2 by 2 factorial experiment design was used. A two-way ANOVA model with two main factors imatinib and doxorubicin at day 70, or paclitaxel at day 24, and their interaction term was used. A statistically significant synergistic effect was observed if the interaction term was significant and if its effect was in the same direction.

Results

Foxq1 induces stemness traits and chemoresistance in mammary epithelial cells and breast cancer cells

To determine whether Foxq1 induces stemness traits in mammary epithelial cells, we generated HMLE stable cell model ectopically expressing the Foxq1 gene, in parallel with three known EMT promoting genes Snail, Twist1, and Zeb2 to serve as positive controls (28, 32). As anticipated, the resulting cells (HMLE/Foxq1, HMLE/Twist1, HMLE/Zeb2, and HMLE/Snail) acquired mesenchymal appearances and showed deregulation of epithelial and mesenchymal markers (Supplementary Fig. S1A and S1B). Using flow cytometry, we analyzed cells for CD44 and CD24 expression, two cell-surface markers whose expression in the CD44high/CD24low configuration are associated with both human breast cancer stem cells (CSC) and normal mammary epithelial stem cells (33, 34). More than 90% of the mesenchymal-like cells generated by Foxq1 overexpression acquired the CD44low/CD24low expression pattern, compared with less than 1% of cells expressing control LacZ vector (Fig. 1A). This same cell pattern shift was also observed for the Snail, Twist1, and Zeb2 overexpressed cells (Supplementary Fig. S1C). We then measured the cells’ ability to form mammospheres to confirm acquired stemness. All HMLE cells with these transcription factors formed 8- to 10-fold more mammospheres compared with HMLE cells infected with the corresponding control vector (Fig. 1B and Supplementary Fig. S1D and S1E).

We next investigated the effect of Foxq1 expression on chemoresistance. Using an MTT and clonogenic assay, we observed significantly more surviving HMLE/Foxq1 cells...
A total of 293 genes (99 upregulated genes and 194 downregulated genes) were significantly deregulated (3-fold cut-off level) in HMLE/Foxq1 cells compared with HMLE/LacZ control cells. A detailed analysis of these genes reveals they are involved in different cellular biologic functions including cell-to-cell adhesion, cell motility, EMT, and drug resistance (Fig. 2A and Supplementary Table S3).

Among the downstream targets that are upregulated by Foxq1, PDGFRa and b are two well-known receptors involved in cancer progression (Fig. 2B; refs. 35–38). We therefore explored whether Foxq1 could directly regulate PDGFRa and b promoters. Transcription factor (TF)-binding prediction assays using Vista, CONTRA v2, and the EpiTect ChiP tool revealed seven potential Foxq1 binding sites within the 25-kb PDGFRa promoter region and nine potential Foxq1 binding sites within the 25-kb PDGFRb promoter region (Fig. 2C). By chromatin immunoprecipitation (ChiP)-PCR assay, we demonstrated significant enrichment of DNA for the first Foxq1-binding site (F-P1) in the PDGFRa promoter region and the sixth binding site (F-P6b) in the PDGFRb promoter region, respectively (Fig. 2D). These data were further confirmed by a luciferase assay, where mutations in the core sequence of each of these two binding sites abolished the activation of these two promoters by Foxq1 (Fig. 2E). These results suggest that Foxq1 directly regulates expression of PDGFRa and b.

In parallel, Twist1 and Zeb2 genes were also identified as downstream targets of Foxq1 (Fig. 2A and B), suggesting their role as potential mediating transcription factors for Foxq1. We further identified three potential Foxq1-binding sites in the Twist1 promoter region and two Foxq1-binding sites in the Zeb2 promoter region (Fig. 3A). For the Twist1 promoter, a ChiP-qPCR assay demonstrated enrichment of DNA in the first binding site (F-T1) close to the transcription start site (Fig. 3B). For the Zeb2 promoter, ChiP-qPCR data also showed that the first binding site (F-Z1) was significantly enriched in the precipitated protein–DNA complex (Fig. 3B). The results of ChiP-qPCR were further

The transcriptional axis between Foxq1, Twist1, and PDGFRs and their clinical correlation

To gain more insight into the mechanism of Foxq1, we performed expression profiling to identify downstream targets of Foxq1. A total of 293 genes (99 upregulated genes and 194 downregulated genes) were significantly deregulated (3-fold cut-off level) in HMLE/Foxq1 cells compared with HMLE/LacZ control cells. A detailed analysis of these genes reveals they are involved in different cellular biologic functions including cell-to-cell adhesion, cell motility, EMT, and drug resistance (Fig. 2A and Supplementary Table S3).

Among the downstream targets that are upregulated by Foxq1, PDGFRa and b are two well-known receptors involved in cancer progression (Fig. 2B; refs. 35–38). We therefore explored whether Foxq1 could directly regulate PDGFRa and b promoters. Transcription factor (TF)-binding prediction assays using Vista, CONTRA v2, and the EpiTect ChiP tool revealed seven potential Foxq1 binding sites within the 25-kb PDGFRa promoter region and nine potential Foxq1 binding sites within the 25-kb PDGFRb promoter region (Fig. 2C). By chromatin immunoprecipitation (ChiP)-PCR assay, we demonstrated significant enrichment of DNA for the first Foxq1-binding site (F-P1) in the PDGFRa promoter region and the sixth binding site (F-P6b) in the PDGFRb promoter region, respectively (Fig. 2D). These data were further confirmed by a luciferase assay, where mutations in the core sequence of each of these two binding sites abolished the activation of these two promoters by Foxq1 (Fig. 2E). These results suggest that Foxq1 directly regulates expression of PDGFRa and b.

In parallel, Twist1 and Zeb2 genes were also identified as downstream targets of Foxq1 (Fig. 2A and B), suggesting their role as potential mediating transcription factors for Foxq1. We further identified three potential Foxq1-binding sites in the Twist1 promoter region and two Foxq1-binding sites in the Zeb2 promoter region (Fig. 3A). For the Twist1 promoter, a ChiP-qPCR assay demonstrated enrichment of DNA in the first binding site (F-T1) close to the transcription start site (Fig. 3B). For the Zeb2 promoter, ChiP-qPCR data also showed that the first binding site (F-Z1) was significantly enriched in the precipitated protein–DNA complex (Fig. 3B). The results of ChiP-qPCR were further
con
fi
rmed in luciferase assays in a dose- and sequence-dependent manner (Fig. 3C and D).

As Twist1 and Zeb2 downstream targets, the expression of PDGFRs was validated by RT-PCR in Twist1- and Zeb2-overexpressing model cell lines (Supplementary Fig. S3A and S3B). In addition, we found five Twist1-binding sites within the PDGFRα promoter region, and ten in the β promoter region, respectively (Supplementary Fig. S3C). For the PDGFRα gene, ChIP-qPCR indicated the enrichment of DNA in only the second Twist1-binding site (T-Pα2). For the PDGFRβ gene, Twist1 binding was

Figure 2.
Foxq1 directly regulates PDGFRs. A, heatmap showing expression levels of the selected genes in HMLE/LacZ and HMLE/Foxq1 cells. Data are normalized to GAPDH expression. Log2 intensity scale is shown on right. B, the relative expression levels of the Twist1, Zeb2, and PDGFRα and β genes in HMLE cells with Foxq1 or control LacZ overexpression was measured by real-time RT-PCR assay (**, P < 0.01; *** P < 0.001). C, potential binding sites of Foxq1 in the promoter regions of PDGFRα and β genes were identified by an in silico analysis. D, ChIP-qPCR analysis showed enrichment of the binding DNA in binding sites for PDGFRα (F-Pα1) and PDGFRβ (F-Pβ6; ***, P < 0.01). Dotted line represents 3-fold enrichment. E, the binding of Foxq1 to the PDGFRα and β promoter region was confirmed by luciferase assay. The Foxq1 conservative binding sequence (WT) and mutant sequence (MT) in PDGFRα and β promoters was shown (***, P < 0.001).

Figure 3.
Foxq1 directly regulates Twist1 and Zeb2. A, potential binding sites of Foxq1 in the promoter regions of Twist1 and Zeb2 genes were identified by an in silico analysis. B, ChIP-qPCR analysis showed enrichment of the binding DNA in binding sites for Twist1 (F-T1) and Zeb2 (F-Z1; **, P < 0.01). Dotted line represents 3-fold enrichment. C, luciferase assay showed Foxq1 activated the Twist1 (left) and Zeb2 (right) promoter in a dose-dependent manner (*, P < 0.05; **, P < 0.01; and ***, P < 0.001). D, the binding of Foxq1 to the Twist1 and Zeb2 promoter regions was confirmed by luciferase assay. The Foxq1 conservative binding sequence (WT) and mutant sequence (MT) are shown (***, P < 0.001).
observed in the first binding site (T-P\(b\)1) (Supplementary Fig. S3D). These results were further validated by luciferase assays (Supplementary Fig. S3E and S3F). Although six Zeb2-binding sites were predicted to exist in the PDGFR\(b\) promoter region, none of these sites showed significant Zeb2 enrichment by ChIP-PCR (Supplementary Fig. S3C and S3D). Taken together, these data strongly suggest an upstream role for Foxq1 on Twist1 and Zeb2 genes, and that Foxq1 controls PDGFR\(a\) and \(b\) expression through both direct and indirect mechanisms.

We next analyzed the expression correlation of these genes in The Cancer Genome Atlas (TCGA) database. The expression of Foxq1 and Twist1 are closely correlated with PDGFR\(a\) and \(b\) (Supplementary Fig. S4A) in the level 3 gene expression (RNA-seqV2) dataset for breast cancer. More importantly, the correlation of Foxq1, Twist1, and PDGFRs significantly correlated with a poor prognosis (Supplementary Fig. S4B). A similar correlation was also observed in uterine corpus endometrial carcinoma and several different cancer types (Supplementary Fig. S4C and data not shown). This discovery substantiates the clinical importance of the transcriptional cascade of Foxq1/Twist1/PDGFRs in cancer progression.

Knockdown of PDGFRs blocks Foxq1-promoted oncogenesis in vitro and in vivo

To investigate the role of PDGFR\(a\) and \(b\) in Foxq1-induced oncogenic properties, we knocked down PDGFR\(a\) and \(b\) separately, in HMLE/Foxq1 cells using shRNA technique (Supplementary Fig. S5A and S5B). Silencing of either PDGFR\(a\) or PDGFR\(b\) significantly inhibited cell proliferation (data not shown), cell migration, and invasion (Supplementary Fig. S5C and S5D) when compared with nontarget control counterparts, suggesting that both PDGFR\(a\) and \(b\) contribute to Foxq1-induced cell proliferation and motility.

To explore potential synergistic effects of PDGFR\(a\) and \(b\), we generated a new construct expressing two shRNAs with significant inhibitory effects on both PDGFR\(a\) and \(b\). This construct, when packaged into a lentivirus and infected in HMLE/Foxq1 and HMLER/Foxq1 (HMLE with activated Ras gene and Foxq1) cells, produced significant inhibition of both PDGFR\(a\) and \(b\), which is similar to what was observed for each of the individual shRNAs (Fig. 4A). The basal expression of PDGFRs in HMLE/Foxq1 and HMLER/Foxq1 are comparable (data not shown). As expected, knockdown of both PDGFR\(a\) and \(b\) displayed greater inhibitory effects on cell proliferation, cell migration, and invasion, when compared with knockdown of either PDGFR\(a\) or \(b\) alone (Fig. 4B and C).

To examine the effects of PDGFR\(a\) and \(b\) on Foxq1-induced tumorigenesis and metastasis in vivo, HMLER/Foxq1 cells expressing nontarget control (NT), \(a\), \(b\), or both \(a\) and \(b\) PDGFR-silencing shRNAs were injected into the fat pads of NCR nu/nu mice. In the absence of PDGFR\(a\) and \(b\), we observed a significant decrease in tumor burdens compared with NT-expressing HMLER/Foxq1 cells, with the double knockdown of \(a\) and \(b\) showing the greatest effect (Fig. 4D). Moreover, HMLER/Foxq1 cells with a NT control showed an average of 16.3 metastatic loci in the lung section. In contrast, cells with either PDGFR\(a\), PDGFR\(b\), or both PDGFRs and \(b\) silencing, showed an average of 7.8, 7, and 3 metastatic loci in the lung section, respectively, as evidenced by positive staining with anti-V5 antibody (Fig. 4E).
Next, we assessed the effects of PDGFRα and β on Foxq1-induced stemness in HMLE cells. We found that knockdown of both PDGFRs, or PDGFRβ alone, resulted in an approximate 25% decrease of CD44high/CD24low cells compared with the nontarget vector control, while knockdown of PDGFRα produced a much smaller effect (<5%; Fig. 5A and Supplementary Fig. S5E). Consistent with these results, knockdown of both PDGFRα and β, or PDGFRβ alone, but not PDGFRα alone, significantly inhibited Foxq1-induced mammosphere formation (Fig. 5B and C and Supplementary Fig. S5F and S5G), suggesting a possible difference of PDGFRβ and PDGFRα in stemness characteristics. This result requires further validation of an in vivo cell dilution assay. Interestingly, knockdown of PDGFRα or β, alone or together, could not reverse Foxq1-induced EMT at the molecular or morphologic level (Fig. 5D and Supplementary Fig. S5H), suggesting Foxq1-induced EMT does not totally depend on PDGFRs.

To explore the clinical implications of the aforementioned discoveries, we investigated the therapeutic effect of the tyrosine kinase inhibitor imatinib, which targets PDGFRs, c-ABL, and Kit (39–41), on Foxq1-driven properties. We found that, in contrast to PDGFRs, there was no significant difference in c-ABL and Kit expression between HMLE/Foxq1 and HMLE/LacZ cells (Supplementary Fig. S6A). Similarly, the phosphorylation level of PDGFRs, but not ABL and Kit, was markedly higher in HMLE/Foxq1 compared with HMLE/LacZ cells (Supplementary Fig. S6B). Furthermore, imatinib treatment led to a significant decrease in the
phosphorylation of PDGFRα and β (Supplementary Fig. S6C), suggesting that PDGFRs are major targets for imatinib in HMLE/Foxq1 cells. We further showed that cell proliferation of HMLE/Foxq1 cells was significantly inhibited with a high-dose imatinib treatment after day 2 (Fig. 6A), whereas cell migration and invasion were markedly inhibited within one day even with low-dose imatinib treatment (Fig. 6B). Moreover, the CD44high/CD24low cell population and mammosphere formation in HMLE/Foxq1 cells were significantly decreased. There is a positive correlation between imatinib concentration and the magnitude of these effects (Fig. 6C and 6D and Supplementary Fig. S6D). Similar results were achieved when we investigated the effects of imatinib treatment on 4T1 cells, which are known to express high level of Foxq1 (data not shown; ref. 26).

Targeting PDGFRs sensitizes Foxq1-overexpressing human mammary epithelial cells and breast cancer cells to chemotherapeutic agents

We next examined the effects of PDGFR on Foxq1-driven chemoresistance. Similar to the effects on stemness traits, knockdown of PDGFRβ showed more significant effects than PDGFRα on sensitizing Foxq1-overexpressing cells to both doxorubicin and paclitaxel, with double knockdown of PDGFRα and β showing the most potent effect (Supplementary Fig. 7).
in contrast to the currently popular notion that EMT is necessarily bound to EMT status. This serendipitous finding is in contrast to the currently popular notion that EMT is a prerequisite for acquiring oncogenic properties during cancer progression, because most EMT promoting genes also promote stemness, oncogenesis, and chemoresistance. However, the results of our study provide intriguing evidence of the disassociation of EMT and oncogenic characteristics in cancer cells, suggesting a more complicated regulatory mechanism in EMT and cancer progression.

We show for the first time that PDGFRα and β have similar functions in oncogenic properties, but have different functions in stemness traits induced by Foxq1 in mammary epithelial cells and breast cancer cells. This result resembles a discovery 30 years ago indicating that PDGFRα and β demonstrate functional specificity during embryogenesis (45, 46). In line with these observations, a recent study showed that PDGFRα, but not PDGFRβ, plays a critical role in invadopodia formation (47). In another study, PDGFRβ, but not PDGFRα, informs intertumoral and intratumoral heterogeneity in glioblastoma (36). Taken together, these studies highlight the convergent and divergent functions of PDGFRα and β in cancer development. The underlying mechanism could be distinct ligand affinities and significant low structure homology in the kinase insert and carboxy-terminal region of these two isoforms, which lead to different downstream signaling activation (48).

More importantly, anti–PDGFR-based approaches sometimes result in a mixed clinical response, suggesting the importance of identifying specific patient subgroups (36, 49). The results of our current study demonstrate that PDGFRs are not only important for the oncogenic properties of cancer cells, but also critical for chemoresistance in human breast cancers overexpressing EMT-promoting genes like Foxq1. These findings provide a rational basis for targeting PDGFRs in conjunction with chemotherapeutic agents for suppressing tumor growth, blocking metastasis or enhancing the efficacy of chemotherapeutic treatments. It is worth to mention that the high doses of imatinib used in our study may not be appropriate for direct transition to the clinic. More specific and less toxic PDGFR inhibitors are needed in this scenario and could be a future direction of our study. In summary, the innovative drug combination we identified provides proof of principle for a novel combinational therapy for patients with breast cancer with aberrant expression of EMT promoting genes such as Foxq1 and Twist1.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed

Authors’ Contributions

Conception and design: F. Meng, G. Wu

Development of methodology: F. Meng, C.L. Speyer, G. Wu

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): F. Meng, F.R. Miller, G. Wu

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.L. Speyer, B. Zhang, Y. Zhao, W. Chen, G. Wu

Writing, review, and/or revision of the manuscript: F. Meng, C.L. Speyer, B. Zhang, D.H. Gorski, F.R. Miller, G. Wu

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): F. Meng, Y. Zhao, G. Wu

Study supervision: G. Wu

Acknowledgments

The authors thank Dr. Robert A. Weinberg at the Massachusetts Institute of Technology (Boston, MA) for providing the HMLE and HMLER cell lines. The authors also thank Ms. Elizabeth A. Katz of the Karmanos Cancer Institute of Detroit and Ms. Elizabeth A. Katz of the Karmanos Cancer Institute of Detroit.
Cancer Institute's Marketing and Communications Department for editing the manuscript.

**Grant Support**

This work was supported by ACS IRG grant from the Karmanos Cancer Institute, Wayne State University (G. Wu), and NIH/NCI grant ROI CA163772 (B. Zhang). The Biostatistics Core and the Bioinformatics Core of the Karmanos Cancer Institute are supported by grant number P30-CA22453-29.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 23, 2013; revised October 10, 2014; accepted November 9, 2014; published OnlineFirst December 10, 2014.

**References**

3. Shah AN, Gallick GE. Src, chemoresistance and epithelial to mesenchymal transition, are they related? Anticancer Drugs 2007;18:
10. Kurrey NK, Jalgaonkar SP, Joglekar AV, Ghanate AD, Chaskar PD, Doi–

Downloaded from cancerres.aacrjournals.org on April 19, 2017. © 2015 American Association for Cancer Research.
PDGFR\(\alpha\) and \(\beta\) Play Critical Roles in Mediating Foxq1-Driven Breast Cancer Stemness and Chemoresistance

Fanyan Meng, Cecilia L. Speyer, Bin Zhang, et al.

Cancer Res 2015;75:584-593. Published OnlineFirst December 10, 2014.

Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-13-3029

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2014/12/10/0008-5472.CAN-13-3029.DC1

Cited articles
This article cites 49 articles, 18 of which you can access for free at:
http://cancerres.aacrjournals.org/content/75/3/584.full.html#ref-list-1

Citing articles
This article has been cited by 4 HighWire-hosted articles. Access the articles at:
/content/75/3/584.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.