FOXO1 Inhibits Runx2 Transcriptional Activity and Prostate Cancer Cell Migration and Invasion

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Abstract

Prostate cancer patients with regional lymph node involvement at radical prostatectomy often experience disease progression to other organs, with the bone as the predominant site. The transcription factor Runx2 plays an important role in bone formation and prostate cancer cell migration, invasion, and metastasis. Here we showed that the forkhead box O (FOXO1) protein, a key downstream effector of the tumor suppressor PTEN, inhibits the transcriptional activity of Runx2 in prostate cancer cells. This inhibition was enhanced by PTEN but diminished by active Akt. FOXO1 bound to Runx2 in vitro and in vivo and suppressed Runx2's activity independent of its transcriptional function. FOXO1 inhibited Runx2-promoted migration of prostate cancer cells, whereas silencing of endogenous FOXO1 enhanced prostate cancer cell migration in a Runx2-dependent manner. Forced expression of FOXO1 also inhibited Runx2-promoted prostate cancer cell invasion. Finally, we found that expression of PTEN and the level of FOXO1 in the nucleus is inversely correlated with expression of Runx2 in a cohort of prostate cancer specimens from patients with lymph node and bone metastasis. These data reveal FOXO1 as a critical negative regulator of Runx2 in prostate cancer cells. Inactivation of FOXO1 due to frequent loss of PTEN in prostate cancer cells may leave the oncogenic activities of Runx2 unchecked, thereby driving promiscuous expression of Runx2 target genes involved in cell migration and invasion and favoring prostate cancer progression. Cancer Res; 71(9): 3257–67. ©2011 AACR.

Introduction

Prostate cancer is the most commonly diagnosed malignancy and the second leading cause of cancer deaths in American men. Prostate cancer often metastasizes to other organs and becomes a lethal disease. However, the molecular mechanisms underlying the propensity of prostate cancer to metastasize to long-distance organ sites, particularly to bone, are largely unknown.

The phosphatase and tensin homolog deleted on chromosome 10 (PTEN, also known as MMAC or TEP1) is the most frequently deleted or mutated tumor suppressor gene in human prostate cancers. PTEN is a dual (lipid and protein) phosphatase and functions as tumor suppressor by primarily antagonizing the activity of the PISK/Akt pathway (1). Complete loss (bi-allelic inactivation) of PTEN was detected in 2% to 20% of primary prostate cancer, but up to 60% in metastatic prostate cancer, implying the importance of PTEN inactivation in metastasis of human prostate cancers (2, 3).

Forkhead box O (FOXO) proteins, which include FOXO1 (FKHR), FOXO3a (FKHRL1), FOXO4 (AFX), and FOXO6 in humans, play important roles in regulating many cancer-related functions (4). FOXO proteins primarily function as transcription factors in the nucleus by regulating expression of a large spectrum of tumor suppression genes. Activation of the threonine/serine kinase Akt due to loss of PTEN leads to phosphorylation and nuclear exclusion of FOXO1 (4). Further studies show that FOXO1 plays a crucial role in tumor suppression by acting as a key downstream effector of PTEN (5).

Runt-domain containing protein Runx2 (also called Osf2/Cbfal, AML-3, or Pebp2a) is normally expressed in mesenchymal cells committed to the lineage of osteoblasts. The function of this protein is essential for osteoblast differentiation and maturation and proper bone formation (6, 7). Runx2 can bind to an osteoblast-specific cis-acting element termed OSE2 in the promoter regions of many bone-related factors, including osteocalcin (OC) and osteopontin (OP), thereby transcriptionally upregulating expression of these genes in osteoblasts (8–10).

Expression and function of Runx2 have also been implicated in various human cancers including prostate cancer (11). Increased Runx2 expression positively correlates with Gleason scores and metastasis potential of prostate cancer (12, 13).
Accordingly, Runx2 target genes, including OC, OP, receptor activator of NF-κB ligand (RANKL), osteoprotegerin (OPG), bone morphogenetic proteins (BMP), and VEGF, are well expressed in prostate cancer cells (11, 14, 15). These findings imply that Runx2 is relevant in the development and progression of human prostate cancer. In the present study, we showed that pro-oncogenic activity of Runx2 is negatively regulated by the PTEN/FOXO1 signaling pathway in prostate cancer cells.

Materials and Methods

Cell lines and cell culture

The prostate cancer cell lines PC-3, LNCaP, and DU145 were purchased from American Type Culture Collection (Manassas). The 293T cell line was obtained from Dr. Xianzhen Zhou at University of Minnesota. prostate cancer cells were cultured in RPMI 1640 containing 10% fetal bovine serum (Hyclone), 100 μg/mL streptomycin, and 100 units/mL penicillin. 293T cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum, 100 μg/mL streptomycin, and 100 units/mL penicillin.

Plasmids and small interfering RNAs

Expression vectors for FLAG–FOXO1, FLAG–FOXO1-A3, FLAG–FOXO1–HR–337, FLAG–FOXO1–A3–HR–337, HA–Akt–CA, PTEN and its mutants, and GST–FOXO1 were described previously (16–18). 6XOSE2–Luc and mOG2–Luc reporter constructs and HA-tagged Runx2 expression vector were described previously (19). Renilla luciferase reporter pRL-TK was purchased from Promega, pcDNA3.1 vector was purchased from Invitrogen. The FOXO1 gene-specific short interfering RNA (siRNA; 5’-CCGAAUGCCUAAACAACA-3’), Runx2 siRNA (siGENOME SMARTpool M-012665-01-0005), and nonspecific control siRNA (5’-UAAGCGAUACACACACUA-3’) were purchased from Dharmacon.

Cell transfection and stable cell line generation

Cell transfection was carried out by electroporation as described (20). Transfection efficiencies of 75% to 90% were routinely achieved. For siRNA transfection, cells were transfected with 200-nmol siRNAs specific for FOXO1, Runx2, or nonspecific control siRNA. DU145 Runx2-stable cells (clones #10 and #11) were generated by transfecting DU145 cells with a Runx2 expression vector, followed by G418 (400 μg/mL) selection.

Luciferase reporter assay

For luciferase reporter assays, cells were transfected with firefly luciferase reporter mOG2–Luc or 6XOSE2–Luc plus Renilla luciferase reporter pRL-TK. At 24 hours after transfection, cells were harvested and firefly and Renilla luciferase activities in cell lysates were measured using a dual luciferase kit (Promega). Renilla luciferase activities in cells were used as internal control.

Antibodies, immunoprecipitation, immunoblotting, immunofluorescence cytochemistry, and glutathione S-transferase pull-down assay

The antibodies used were anti-FOXO1, anti-PTEN, anti-Akt (Cell Signaling Technology), anti-FLAG (M2; Sigma-Aldrich), anti-HA (Covance), anti-Rumx2 mouse monoclonal (27-K) rabbit polyclonal (M-70), and anti-ERK2 (Santa Cruz Biotechnology). Protein immunoprecipitations were carried out using an immunoprecipitation kit (Roche Applied Science) as described (17). Immunoblotting, immunofluorescence cytochemistry, and glutathione S-transferase (GST) pull-down assay were conducted as described (17).

Reverse transcription and quantitative real-time PCR

Total cellular RNAs were isolated from cells at 48 hours posttransfection using TRIzol (Invitrogen), and cDNAs were synthesized using SuperScript III reverse transcriptase (Invitrogen). Quantitative real-time PCR was carried out using primers specific for human OP, VEGF, and interleukin 8 (IL8) as described (13). The primers for other genes are matrix metalloproteinase 13 (MMP13), forward 5’-GCACTCTTTTTCTCGGCTTAG-3’ and reverse 5’-CAGGGTCTTGGAGTGGTCA-3’, OC (forward 5’-CACCCTCGCCTATGGACC-3’ and reverse 5’-GCAACTC-GTCACTACCCG-3’), Runx2 (forward 5’-CGGCCCTCTCT-GAACCTC-3’ and reverse 5’-TGCTCTGCTGGGCTCTGA-3’), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), forward 5’-GAAGTGTAAGTGGAGCTGC-3’ and reverse 5’-GAGATGTGATGGAGATTC-3’. Expression levels of genes examined were determined using a SYBR Green Supermix (Bio-Rad) on an iCycler iQ platform (Bio-Rad) according to the manufacturer’s protocol. Reactions were carried out in triplicate and gene expression levels were normalized against the GAPDH gene.

In vitro migration assay

Cells were transfected with indicated plasmids and cultured to confluence on 6-well plates. Cell layer was scratched with a 200-μL pipette tip and detached cells were removed. For each sample, at least 3 scratched fields were photographed immediately and at the time points indicated after the scratch was made using a Leica DMIL inverted microscope and Leica application suite V3 software. Cell migration was evaluated by measuring the difference in wound width.

In vitro invasion assay

In vitro invasion assay was conducted by using BioCoat Matrigel invasion chamber (BD Biosciences) according to the manufacturer’s protocol. Cells were transfected with indicated plasmids, cultured in the insert for 22 hours, and stained with Diff-Quik stain. At least 3 fields for each sample were photographed after invasion. Invasion was evaluated by counting the number of the invaded cells.

Chromatin immunoprecipitation assay

PC-3 cells were transfected with control vector, Runx2 or Runx2 plus FOXO1. At 24 hours after transfection, cells were harvested and subjected to chromatin immunoprecipitation (ChIP) assay as described (21). The soluble chromatin was incubated with 2 μg of anti-Runx2 antibody (Santa Cruz Biotechnology). PCR was carried out using primers specific for the Runx2 binding region in the human OP promoter (forward 5’-GAAATGCACCCCTGCGTAA-3’ and reverse 5’-GGAGGGAGAGATTTGTC-3’) or nonspecific primers.
outside the Runx2 binding region in the OP promoter (forward 5′-ATCTCAAGATGGCTGGGCTC-3′ and reverse 5′-GTCAATT-TAGTGGAGGGAAGTCTG-3′). PCR products were separated on a 2% agarose gel containing ethidium bromide.

Tumor specimens and immunohistochemistry

Seventeen samples of prostate tumors were obtained from the Indiana University Medical Center. All patients had a diagnosis with metastatic prostate cancer. Of 17 samples examined, 8 samples (8/17, approximately 47%) were lymph node metastases, 7 primary prostate cancer samples (7/17, approximately 41%) were obtained from patients with prostate cancer lymph node metastasis, and 2 samples (2/17, approximately 12%) were bone metastases. Sections were cut from formalin-fixed paraffin-embedded tissues, and immunohistochemistry was conducted. Sections were antigen retrieved and immunostaining was conducted as described (20) by using the Elite Avidin-biotin-peroxidase Kit (Vector Laboratories). Antibodies used for immunohistochemistry were anti-PTEN (Cell Signaling Technology; ref 20), anti-FOXO1 (Cell Signaling Technology; ref 22), and anti-Runx2 (27-K, Santa Cruz Biotechnology; ref 13). For scoring of the data, 0 indicates no stain, 1 is low, 2 is intermediate, and 3 is high stain of these proteins.

Statistics

Cell culture experiments were carried out with 3 or more replicates. Statistical analyses were conducted by Student t test. P < 0.05 was considered statistically significant. The nonparametric Spearman rank correlation was used to measure the association of the expression of Runx2, PTEN, and FOXO1 in prostate cancer specimens.

Results

FOXO1 inhibits the transcriptional activity of Runx2 in prostate cancer cells

Activation of the PI3K/Akt pathway is required for the sustained transcriptional activation of Runx2 in both osteoblasts and endothelial cells (23–25). It was reported recently (26) that knockout of the mouse Foxo1 gene in osteoblasts increased expression of OC, a well-studied transcriptional target gene of Runx2 (8, 27). To further test whether FOXO1 inhibition of the OC promoter is mediated through Runx2, we examined the effect of FOXO1 on a composite reporter gene (6XOSE2-Luc) which contains 6 copies of the Runx2 responsive element OSE2 derived from the mouse OC gene. This reporter gene was active in PC-3 cells transfected with Runx2 (Fig. 1C). However, this activity was abrogated in cells cotransfected with FOXO1 (Fig. 1C). These data suggest that FOXO1 inhibition of the OC promoter activity is mediated through Runx2.

FOXO1-mediated inhibition of Runx2 is regulated by the PTEN/Akt pathway

PC-3 cells do not express a functional PTEN (20, 28) and loss of PTEN leads to Akt activation and cytoplasmic localization of FOXO1 (4). As expected, wild-type FOXO1 (FOXO1-WT) mainly localizes in the cytoplasm of PC-3 cells, whereas the Akt phosphorylation-resistant mutant of FOXO1 (FOXO1-A3) primarily localizes in the nucleus (Supplementary Fig. S1). Forced expression of FOXO1-A3 had a greater inhibitory effect on Runx2 than FOXO1-WT in PC-3 cells (Fig. 1A, lanes 6, 5), suggesting that FOXO1 inhibition of Runx2 can be regulated by the PTEN/Akt pathway. Indeed, forced expression of wild-type PTEN enhanced FOXO1 inhibition of Runx2 in LNCaP cells (Fig. 1B left, lanes 3, 2). Expression of PTEN alone also inhibited Runx2 (Fig. 1B left, lanes 7, 1). This effect of PTEN is presumably mediated through endogenous FOXO1 which is readily expressed (Fig. 1B, left). As illustrated in Fig. 1B, the PTEN mutants C124S and G129R lack both lipid and protein phosphatase activity but the G129E mutant retains protein phosphatase function (29). Different from wild-type PTEN, however, none of the PTEN mutants was able to enhance FOXO1-mediated inhibition of Runx2 (Fig. 1B left, lanes 4–6). Similar to the effect of C124S and G129R, expression of the G129E mutant also failed to inhibit Runx2 in LNCaP cells without transfection of FOXO1 (Fig. 1B left, lanes 8–10). These data suggest that the lipid, but not protein phosphatase activity of PTEN may be important for its function in enhancing FOXO1-mediated inhibition of Runx2. If this were true, we would anticipate that FOXO1-mediated inhibition of Runx2 is diminished or blocked by Akt, which is activated due to loss of PTEN’s lipid phosphatase activity. Ectopic expression of FOXO1 inhibits Runx2 in PTEN-positive DU145 cells stably transfected with Runx2 (Fig. 1D). However, this effect of FOXO1 was diminished by forced expression of constitutively active Akt, Akt-CA (Fig. 1D). Together, these data indicate that FOXO1 inhibition of Runx2 is regulated positively by PTEN but negatively by Akt in prostate cancer cells.

FOXO1 inhibits Runx2 in a manner independent of its transcriptional activity

While FOXO1 primarily functions as a DNA-binding transcription factor (4), increasing evidence indicates that FOXO1 also possesses a transcription-independent function by interacting with other transcription factors, which
Figure 1. FOXO1 inhibits the transcriptional activity of Runx2. A, effect of FOXO1-WT and FOXO1-A3 on Runx2. PC-3 cells were transfected with a luciferase-based OC promoter reporter (mO2G2-Luc), a Renilla luciferase reporter and plasmids as indicated. At 24 hours after transfection, luciferase activities were measured. Error bars, SD among 3 individual experiments. *, P < 0.05. Expression of FOXO1 and Runx2 proteins was analyzed by immunoblotting (IB). ERK2 was used as a loading control. B, left, effect of wild-type and mutated PTEN on Runx2. LNCaP cells were transfected with plasmids as indicated and luciferase activities were measured and analyzed as described in (A). *, P < 0.05; #, P > 0.05. Right, a diagram summarizing the lipid and protein phosphatase activity of wild-type and mutated PTEN. C, effect of FOXO1 on 6XOSE2-Luc. PC-3 cells were transfected with 6XOSE2-Luc, a Renilla luciferase reporter and plasmids as indicated. Luciferase activities were measured as described in (A). *, P < 0.05. D, effect of Akt on FOXO1 inhibition of Runx2. DU145 Runx2-stable cells (clone #10) were transfected with the plasmids as indicated and luciferase activities were measured as described in (A). *, P < 0.05.
include the androgen receptor and the estrogen receptor, among others (18, 30–36). Mutation of the histidine 215 residue to arginine (HR) in the DNA binding domain of FOXO1 abolishes binding of FOXO1 to the forkhead responsive element in its target genes (37). Deletion of a transactivation domain in the COOH-terminus of the FOXO1 protein (amino acids 538–655) also abrogates the transcriptional activity of FOXO1 (38). Ectopic expression of FOXO1 transcription-deficient mutant FOXO1-HR-537 inhibited Runx2-induced activation of the OC promoter in a degree similar to FOXO1-WT (Fig. 2A). Thus, FOXO1 inhibits Runx2 in a manner independent of its transcriptional activity.

FOXO1 interacts with Runx2 in vitro and in prostate cancer cells

Having shown that FOXO1 inhibits Runx2 without the need of its DNA-binding function, we sought to determine whether FOXO1 physically interacts with Runx2. FLAG–FOXO1 and

![Figure 2](https://www.aacrjournals.org/cancerres/article-fig/71/9/3261/F2.html)
HA–Runx2 were cotransfected into 293T cells and cell lysates were subjected to immunoprecipitation. Ectopically expressed HA–Runx2 was detected in the protein complexes immunoprecipitated by the anti-FLAG antibody (Fig. 2B). Reciprocally, FLAG–FOXO1 was detected in the protein complexes immunoprecipitated by the anti-HA antibody. FLAG–FOXO1 and HA–Runx2 proteins are also associated with each other in DU145 cells (Fig. 2C, left). Coimmunoprecipitation assays showed that endogenous FOXO1 proteins interact with Runx2 in the DU145 Runx2-stable cell line (Fig. 2C, right). To further determine whether FOXO1 physically interacts with Runx2, 5 GST–FOXO1 fusion proteins were purified (Fig. 2D, top and bottom) and incubated with lysates of DU145 cells transfected with HA–Runx2. As shown in Fig. 2D (middle), Runx2 proteins were specially pulled down by GST–FOXO1 fusion protein FO1–2, which contains amino acids from 149 to 267 in FOXO1. The same region of FOXO1 has been shown to interact with other proteins such as cyclin D1 (39), suggesting that FO1–2 may act as a functional motif that interacts with other proteins. Collectively, both coimmunoprecipitation and GST pull-down assay showed that FOXO1 interacts with Runx2 in vitro and in prostate cancer cells.

**FOXO1 inhibits expression of endogenous genes regulated by Runx2**

Runx2 plays an important role in cancer metastasis by regulating expression of a large spectrum of genes that are involved in vessel formation, invasion, extravasation, and colonization (11). These genes include, but are not limited to, OP, IL8, VEGF, and MMPs (13). Transfection of Runx2 in LNCaP prostate cancer cells increased expression of endogenous OP, IL8, VEGF, and MMP13 genes (Fig. 3A). However, this activity of Runx2 was diminished by FOXO1 (Fig. 3A). FOXO1 also inhibited expression of endogenous OC in PC-3 cells (Fig. 3B). Knockdown of endogenous Runx2 decreased OC expression in PC-3 cells that express relatively high levels of Runx2 (Supplementary Fig. S2). However, no significant
inhibition of FOXO1 on OC expression was observed in PC-3 cells treated with Runx2 siRNAs (Fig. 3B, lanes 4, 3), suggesting that FOXO1 inhibition of OC expression is mediated through endogenous Runx2 in PC-3 cells.

We also examined whether expression of Runx2 target genes is affected by endogenous FOXO1. Having shown that the inhibitory effect of FOXO1 on Runx2 is markedly increased by PTEN expression (Fig. 1B), we chose PTEN-positive DU145 Runx2-stable cells (clone #10) to assess the effect of endogenous FOXO1 on Runx2 activity. The basal levels of VEGF and IL8 were detectable in the DU145 Runx2-stable cells (Fig. 3C). Importantly, knockdown of endogenous FOXO1 increased expression of VEGF and IL8 (Fig. 3C). However, this effect of FOXO1 silencing was abolished by Runx2 knockdown (Fig. 3C). Similar results were obtained in another independent Runx2-stable clone (clone #11) of DU145 cells (data not shown). Thus, expression of Runx2 target genes is negatively regulated by endogenous FOXO1 in prostate cancer cells.

OP is a well-characterized target gene of Runx2 (40, 41). There is a defined Runx2 binding element OSE2 in its promoter...
The binding of Runx2 to the OP (Fig. 3D, lanes 4, 10), suggesting that the binding of Runx2 to the OP promoter was detected in the region containing the OSE2 site, but not the region outside this site in PC-3 cells (Fig. 3D, lanes 4, 10), suggesting that the binding of Runx2 to the OP promoter detected by the anti-Runx2 antibody is specific. The binding of Runx2 to the OP promoter increased in cells transfected with Runx2 (Fig. 3D, lanes 11, 10). This effect of Runx2 was abolished by cotransfection of FOXO1 (Fig. 3D, lanes 12, 11). These data suggest that FOXO1 inhibits the binding of Runx2 to the promoter of its target gene OP in PC-3 cells.

FOXO1 inhibits Runx2-induced migration and invasion of prostate cancer cells

Runx2 promotes prostate cancer cell migration and invasion (13, 42). We examined whether FOXO1 affects Runx2-mediated prostate cancer cell migration and invasion using wound healing and transwell Matrigel invasion assays. Expression of Runx2 enhanced migration of PC-3 cells (Fig. 4A and B, columns 1, 4). This effect of Runx2 was abolished by cotransfection of FOXO1-WT and FOXO1-A3 (Fig. 4A and B, column 5, 6 versus 4). Transfection of PC-3 cells with FOXO1-WT or FOXO1-A3 alone also inhibited PC-3 cell migration (Fig. 4A and B, column 2, 3 versus 1). Knockdown of endogenous FOXO1 in DU145 Runx2-stable cells increased cell migration (Fig. 4C and D, columns 2, 1). This effect was completely blocked by siRNA-mediated depletion of Runx2 (Fig. 4C and D, columns 3, 2). Knockdown of Runx2 alone decreased the migration of these cells (Fig. 4C and D, columns 4, 1). Thus, silencing of endogenous FOXO1 increased prostate cancer cell migration in a Runx2-dependent manner.

Matrigel invasion assay was employed to determine whether FOXO1 negatively regulates Runx2-induced invasion of prostate cancer cells. Invasive ability of PC-3 cells transfected with FOXO1-A3 was lower than mock transfected cells (Fig. 5A, upper middle). Similar to the previous report that Runx2 is critical for invasion of PC-3 cells, overexpression of Runx2 markedly enhanced PC-3 invasion (Fig. 5A, lower left). This effect of Runx2 was inhibited in cells transfected with FOXO1-A3 (Fig. 5A, lower middle). Consistent with the finding that transactivation-deficient mutant of FOXO1 inhibited the transcription activity of Runx2 (Fig. 2A), expression of FOXO1-A3–HR-537 inhibited invasive ability of PC-3 cells (Fig. 5A, upper right). Runx2-enhanced invasion of PC-3 cells was also blocked by this mutant (Fig. 5A, lower right). The quantitative data from 3 independent experiments are presented in Fig. 5B. Thus, Runx2-promoted invasion of PC-3 cells can be inhibited by FOXO1 and this effect of FOXO1 is independent of its transcriptional activity.

Expression of PTEN and the level of FOXO1 in the nucleus inversely correlate with Runx2 expression in prostate cancer specimens from patients with lymph node or bone metastasis

To further investigate the relevance of negative regulation of Runx2 by the PTEN/FOXO1 pathway in prostate cancer, we examined by immunohistochemistry the expression of PTEN, FOXO1, and Runx2 proteins in a cohort of 17 prostate cancer specimens obtained from patients with either lymph node or bone metastasis. As summarized in Fig. 6A, approximately 65% (11/17) of cases strongly expressed Runx2 protein. Expression of PTEN protein was not detected in approximately 35% (6/17) of cases, and approximately 41% (7/17) of cases exhibited lower or intermediate expression of PTEN (Fig. 6A). Approximately 30% (5/17) of prostate cancer specimens expressed lower or intermediate levels of FOXO1 proteins in the nucleus. No expression of FOXO1 protein in the nucleus was detected in the rest (12/17, approximately 70%) of the specimens examined (Fig. 6A).

Examples for both positive and negative staining of Runx2, PTEN, and FOXO1 proteins can be seen in Fig. 6B and C. There is an inverse correlation between Runx2 and PTEN expression (the correlation is $-0.77, P < 0.001$). Expression of Runx2 and the level of FOXO1 in the nucleus is also inversely correlated (the correlation is $-0.47$). The correlation approaches (but does not reach) statistical significance ($P = 0.059$). One of the factors causing this could be the small size of samples analyzed. The correlation between PTEN and nuclear expression of FOXO1 is 0.54, and the correlation is statistically significant ($P < 0.05$).
Discussion

Prostate cancer dissemination represents a major therapeutic challenge. It is urgent to understand the molecular basis responsible for the steps involved in prostate cancer metastasis, such as cell invasion and migration. By using PC-3 cells as a working model, previous studies showed that Runx2 is an important factor in regulating prostate cancer cell migration, invasion, and metastasis (13, 27, 42). Here we showed that FOXO1 binds to and inhibits the transcriptional activity of Runx2 in various prostate cancer cell lines. Forced expression of Akt can reverse FOXO1-mediated inhibition of Runx2 in PTEN-positive DU145 cells. In contrast, FOXO1 inhibition of Runx2 was largely enhanced by PTEN expression in PTEN-null LNCaP cells. Moreover, expression of the Akt phosphorylation-resistant mutant FOXO1-A3 resulted in a much greater inhibitory effect on Runx2 than the wild-type counterpart. We further showed that Runx2-promoted migration and invasion of prostate cancer cells is inhibited by FOXO1. Thus, we provide evidence that FOXO1 is a key negative regulator of Runx2 as well as prostate cancer cell migration and invasion. This function of FOXO1 is abrogated by Akt activation or PTEN loss.

During disease progression human prostate cancers often metastasize to lymph node and bone. The PTEN tumor suppressor gene is implicated in this process because it is highly deleted or mutated in advanced/metastatic prostate cancer (3). Surprisingly, mouse genetic studies demonstrate that although homozygous deletion of the PTEN gene in the prostate inevitably promotes primary tumor formation, the frequency of PTEN-knockout tumors to metastasize to other organs, such as lymph node and bone, is very low or none (43–45). One possible interpretation for this enigma could be that loss of PTEN may need to cooperate with other “hits” as joined
forces for prostate cancer cells to metastasize. In agreement with other reports (46), we showed here that expression of PTEN protein is frequently reduced or lost in a cohort of prostate cancer patients with lymph node and bone metastasis. Importantly, we found that loss of PTEN occurs concurrently with overexpression of Runx2, a factor that is implicated in prostate cancer metastasis (11). Based upon these findings, we envision a model (Fig. 6D) wherein Runx2-mediated gene transcription and cell migration and invasion are limited due to FOXO1 inhibition of Runx2 in PTEN-positive prostate cells. In PTEN-null prostate cancer cells, however, activation of Akt leads to FOXO1 phosphorylation and nuclear exclusion, which in turn abolishes FOXO1-mediated inhibition of Runx2 and thereby favors Runx2-mediated gene transcription and prostate cancer cell migration and invasion. Thus, PTEN loss and Runx2 overexpression may function as 2 key factors that work in concert in promoting human prostate cancer metastasis. Investigation of this hypothesis with animal models is warranted.

In summary, we show here that the transcriptional activity of Runx2 is negatively regulated by FOXO1 in prostate cancer cells. This effect of FOXO1 is enhanced by PTEN but inhibited by Akt. Our further analysis demonstrates that loss of PTEN and Runx2 overexpression occur concurrently in a cohort of patients with lymph node and bone metastatic prostate cancer. Thus, we identify FOXO1 as a key negative regulator of Runx2 function. FOXO1 inactivation due to frequent loss of PTEN in advanced prostate cancer may favor metastasis and progression of prostate cancers, especially those with Runx2 overexpression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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