Review

Cancer Research

FOXM1 in Cancer: Interactions and Vulnerabilities

Andrei L. Gartel

Abstract

FOXM1 is a transcription factor of the Forkhead family that is required for cell proliferation of normal cells. However, FOXM1 is repeatedly overexpressed in a variety of human cancers, and it has been implicated in all major hallmarks of cancer delineated by Hanahan and Weinberg. It has been postulated that the oncogenic potential of FOXM1 is determined by its capacity to transactivate target genes that are implicated in different phases of cancer development. However, FOXM1 may also play an oncogenic role by interacting with other proteins, such as β-catenin or SMAD3 to induce oncogenic WNT and TGFβ signaling pathways, respectively. In this review, I will discuss the protein–protein interactions of FOXM1 that are critical for cancer development and may represent novel targets for anticancer drugs. *Cancer Res; 77(12); 3135–9. ©2017 AACR.*

Downloaded from http://aacrjournals.org/cancerres/article-pdf/77/12/3135/2748374/3135.pdf by guest on 11 August 2024

Introduction

The mammalian transcription factor Forkhead Box M1 (FOXM1) is one of the members of the Forkhead family proteins that share a 100 amino acid long winged-helix DNA-binding domain (1). There are four FOXM1 isoforms: FOXM1A, B, C, and D generated by the alternative splicing. FOXM1A is a transcriptional repressor that locates in cytoplasm. FOXM1C is a transcriptional activator overexpressed in some tumors, and FOXM1D predominantly locates in cytoplasm and may promote metastasis. In this review, we are focused on FOXM1B that we refer as FOXM1. We believe that focus on FOXM1B is justified as FOXM1B (FOXM1), but not other isoforms, is dominantly overexpressed in human cancers and may be an attractive target for anticancer drugs. Overexpression of FOXM1 has been detected in a broad range of cancer types, suggesting that FOXM1 is essential for tumorigenesis (1–3). Although in normal cells, FOXM1 is mainly responsible for cell proliferation (3), in cancer cells, it contributes to all hallmarks of cancer described by Hanahan and Weinberg (3, 4). Suppression of FOXM1 in tumor cells by RNAi led to reduced cell proliferation, anchorage-independent growth (5) migration, invasion and angiogenesis (reviewed in ref. 3), suggesting that FOXM1 regulates these properties of cancer cells. In addition, FOXM1 evades the action of the tumor suppressor p53. We and others showed that FOXM1 expression increased after mutating or deleting p53 (6, 7), suggesting that p53 inactivation in human tumors leads to the overexpression of FOXM1. FOXM1 also increases the resistance of cancer cells to apoptosis and genomic instability (3).

Recently, it has been shown that the FOXM1-regulatory network is a major predictor of adverse outcomes in 18,000 cancer cases across 39 human malignancies (8), confirming the important role of FOXM1 in cancer. However, it is still not fully understood how FOXM1 exerts its oncogenic activity in human cells. One mechanism is linked to the transcriptional activation of FOXM1 targets that leads to several protumorigenic effects, including enhanced cell proliferation (3). Alternatively, FOXM1 may act as an oncogene by interacting with other proteins, thus supporting distinct oncogenic pathways. In this review, I will describe the known interactions of FOXM1 in cancer, and I will discuss how this information may help in developing novel specific FOXM1 inhibitors.

Interaction between NPM and FOXM1 is required for FOXM1 expression and may explain the outcome of AML treatment

Using mass spectrometric analysis, we found that FOXM1 interacts with the multifunctional protein nucleophosmin (NPM) that shuttles between the nucleus and cytoplasm and is overexpressed in a variety of human tumors (3, 5). Immunofluorescence microscopy confirmed the colocalization of FOXM1 and NPM in the nucleus of human cancer and immortal cells. Furthermore, knockdown of NPM in immortal and cancer cells led to significant downregulation of FOXM1, suggesting that NPM might stabilize FOXM1 protein.

In a groundbreaking research article published in 2005 (9), it was shown that in 30% of acute myeloid leukemia (AML) patients, *NPM1* gene is mutated in exon 12, leading to relocalization of NPM protein to the cytoplasm and to significantly better prognosis for AML patients after chemotherapy. However, the explanation to this phenomenon was lacking. We found that in the AML cell line OCI-AML3 with *NPM1* exon 12 mutation (10), FOXM1 protein colocalized with NPM in the cytoplasm (5). We hypothesized that the improved outcomes in this subset of $NPM1^{mut}$ AML are associated with the cytoplasmic localization and consequent inactivation of oncogenic transcription factor FOXM1. To prove this notion, we demonstrated predominantly cytoplasmic localization of FOXM1 and NPM only in AML primary patient samples with NPM1 mutations (11). In addition, we investigated the effects of nuclear FOXM1 on chemoresistance to cytosine arabinoside (cytarabine), the backbone of AML chemotherapy, and we found that stable knockdown of FOXM1 in AML cell lines with nuclear FOXM1 resulted in increased sensitivity to this chemotherapeutic agent (11).

Residues 187-295 AA of NPM have been identified as essential for the interaction with FOXM1 (5). We investigated

Department of Medicine, University of Illinois at Chicago, Chicago, Illinois.

Corresponding Author: Andrei L. Gartel, Department of Medicine, University of Illinois at Chicago, 840 S. Wood St., Chicago, IL 60612. Phone: 312-996-1855; Fax: 312-413-0342; E-mail: agartel@uic.edu

doi: 10.1158/0008-5472.CAN-16-3566

©2017 American Association for Cancer Research.

whether the truncated NPM corresponding to 187-295 AA inhibits the interaction of full-length NPM and FOXM1 (365-748 AA). In NMR experiments, we observed a reduction in the chemical shift changes upon the addition of this truncated NPM, indicating that the FOXM1/NPM interaction was significantly inhibited. These data suggested that an NPM peptide that interacts with FOXM1 competes with NPM for FOXM1 binding and inhibits FOXM1/NPM interaction, and FOXM1 expression in human cancer cells. Overall, in cancer cells, NPM interacts with FOXM1, and their interaction is required for sustaining the level and localization of FOXM1 (5). In AML cells, mutant NPM drives FOXM1 to the cytoplasm, leading to its inactivation (11). Generally speaking, the interaction of nuclear FOXM1 with NPM may be targeted by peptides or small molecules in human cancer.

MELK binds and activates FOXM1 in glioma cells

Maternal embryonic leucine-zipper kinase (MELK) is a serine/threonine kinase that is overexpressed in several human cancers, including glioma (12). MELK directly binds to FOXM1 and regulates its phosphorylation and activation in glioma stem-like cells (13). Interestingly, that activation of FOXM1 was also dependent on the binding and phosphorylation by PLK1 kinase, because the FOXM1 mutant that could only bind PLK1 but could not get phosphorylated by it, and it was not activated by MELK (13). Using mouse neural progenitor cells, it has been found that overexpression of FOXM1 accelerated neurosphere formation, while FOXM1 expression increased as cells progressed to pretumorigenic progenitors and glioma stem-like cells (13). The authors suggested that FOXM1 is involved in the development of glioblastoma multiforme, and MELK–FOXM1 interaction is a potential therapeutic target in glioma (13). Unfortunately, the drug siomycin A, which the authors of this work used to illustrate the targeting of FOXM1/MELK, did not inhibit FOXM1/MELK interaction, but inhibits FOXM1 expression as a proteasome inhibitor (14, 15). Therefore, it is too early to predict whether the strategy to target FOXM1/MELK interaction with objection to inhibit cancer growth will be successful.

Pin1–FOXM1 interaction induces metastatic melanoma

Pin1 is a phospho-specific peptidyl-prolyl isomerase that facilitates substrate isomerization through interaction with phosphorylated Ser/Thr-Pro motifs. In malignant melanoma, FOXM1 activity correlated with Pin1 expression (16). Moreover, Pin1 acted as the main regulator of FOXM1 activity in the melanoma cells through physical binding with FOXM1, and the driver of melanoma, oncogenic BRAFV600E, stimulated this interaction. Depletion of FOXM1 by RNAi in melanoma cells or inhibition of binding by cell-permeable Pin1–FOXM1-blocking peptides repressed FOXM1 activity and inhibited melanoma cell proliferation *ex vivo* (16). These data suggest that the physical interaction of FOXM1–Pin1 may increase FOXM1 activity in melanoma cells, and Pin1–FOXM1 inhibitors may work as anticancer drugs in melanoma.

STAT3 interacts with FOXM1 in glioblastoma after radiation treatment

Radiation of glioblastoma cells led to the phosphorylation of STAT3 and the induction of FOXM1. Increase of FOXM1 expression was linked to the direct interaction of FOXM1 with STAT3 (17). Colocalization of FOXM1 and STAT3 in the nucleus of radiation-treated cells was also confirmed by immunofluorescence (17), and suppression of STAT3 repressed FOXM1 expression. In addition, FOXM1 inhibition sensitized glioblastoma cells to radiation. The authors suggested that the FOXM1/STAT3 interaction may contribute to the activation of FOXM1 expression and resistance to radiation in glioma stem cells (17).

Interaction of p65 with FOXM1/β-catenin is critical in CML

It has been shown that NF-κB (p65) protein physically interacts with FOXM1/β-catenin and transcriptionally induces FOXM1 expression in chronic myelogenous leukemia (CML) cells (18). At the same time, β-catenin activation promotes nuclear translocation of p65. Silencing of FOXM1 led to the suppression of p65 and β-catenin, whereas silencing of β-catenin led to the suppression of FOXM1 and p65 (18). These data indicate mutual positive regulation between p65 and FOXM1/β-catenin in CML. Disruption of the p65-FOXM1/β-catenin positive feedback loop in CML cells impaired the self-renewal capacity and survival of CML stem cells (18). These data suggest that p65–FOXM1 interaction has oncogenic activity in CML cells.

The long noncoding RNA PVT1 binds FOXM1 protein and posttranslationally stabilizes FOXM1 protein

LncRNA-PVT1 is overexpressed in different cancer types and may promote tumor growth and metastasis in gastric cancer (19). Using RNA–protein pull-down assay, it has been shown that PVT1 directly binds to FOXM1 protein and inhibits its degradation in gastric cells (19). In addition, FOXM1 binds to the PVT1 promoter and activates its transcription. *In vitro* metastasis assays showed that FOXM1 knockdown partially diminished the effects of PVT1 overexpression on gastric cancer cell metastasis. Similarly, it has been demonstrated that FOXM1 knockdown could, to some extent, abolish the increased tumor size initiated by the overexpression of PVT1 in mouse xenograft models (19). These data do not give a clear answer whether PVT1 or FOXM1 is the effector or the stabilizing cofactor of tumor growth and metastasis in gastric cancer. Additional experiments are required to resolve this question.

FOXM1 determines β-catenin nuclear localization in glioma cells

Wnt signaling is the primary signaling system in stem/progenitor and cancer cells that leads to the stabilization of β-catenin. The transcriptional complex of β-catenin with TCF/LEF in the nucleus activates the expression of Wnt target genes important for cancer development (20). Activation of β-catenin has been implicated in the development of glioblastoma multiforme, the most malignant form of glioma (21). It turned out that FOXM1 directly binds to β-catenin in the nucleus of glioma cells and enhances β-catenin transcriptional activity (22). Deletion of FOXM1 or use of FOXM1 mutants that abolish interaction with β-catenin prevented β-catenin nuclear localization and inhibited Wnt signaling in glioma cells (22). Conversely, knockdown of β-catenin in glioma cells diminished their tumorigenicity, indicating that the oncogenic activity of FOXM1 in glioma depends on β-catenin. The authors of this work suggested that FOXM1 regulates two facets of β-catenin nuclear function: nuclear accumulation and assembly of a transcription activation complex. In contrast to the majority of other cancers where FOXM1 directly induces oncogenesis as a transcription factor, in glioma, FOXM1 contributes to tumorigenesis via

Downloaded from http://aacrjournals.org/cancerres/article-pdf/77/12/3135/2748374/3135.pdf by guest on 11 August 2024

direct interaction with β-catenin, acting as a cofactor that stabilizes β-catenin. These data suggest that direct FOXM1–β-catenin interaction that enhances β-catenin expression and Wnt signaling is required for tumorigenesis in glioma (22).

FOXM1 interacts with SMAD3 and regulates the nuclear retention of the SMAD3/SMAD4 complex in the TGFβ signaling pathway

It has been shown that FOXM1 directly interacts with SMAD3 in breast cancer cells and induces TGFβ/SMAD3–mediated transcription (23). In contrast, the ubiquitin protein ligase transcriptional intermediary factor 1γ (TIF1γ) binds to SMAD4, induces its monoubiquitination, and disruption of the SMAD3/SMAD4 complex (23). Xue and colleagues demonstrated that FOXM1 interferes with SMAD3/TIF1γ interaction and consequently with SMAD4/TIF1γ binding, thus inhibiting the degradation of SMAD4 and stabilizing the SMAD3/SMAD4 complex (23). Moreover, they found that FOXM1/SMAD3 interaction was required for TGFβ-induced breast cancer invasion (24), as a result of SMAD3/SMAD4–dependent transcriptional activation of Slug (Snail2), a C2H2-type zinc finger transcription factor that has been widely expressed in aggressive human cancers (25). Because the inhibitory effects of shFOXM1 on the metastatic ability of breast cancer cells were rescued by the shRNA-resistant FOXM1 R286A/H287A mutant, which is incapable of DNA binding, the effect of FOXM1 on metastasis in breast cancer was not dependent on its transcriptional activity. Instead, FOXM1 acted as a SMAD3-interacting partner that protected SMAD4 from degradation, thus inducing TGFβ signaling and the development of metastasis in breast cancer.

HSP70 inhibits FOXM1 activity and expression

Previously, we showed that proteasome inhibitors suppress FOXM1 transcriptional activity, and as a result of the FOXM1 positive autoregulatory loop (26), they also inhibit FOXM1 mRNA and protein expression (14, 15). Later, we demonstrated that HSP90 inhibitor PF-4942847 and heat shock also suppress FOXM1 in several human cancer cell lines (27). It turned out that all of these treatments have something in common: They all induce HSP70. Moreover, when we used HSP70 inhibitor 9AA or HSP70-siRNA in combination with proteasome/HSP90 inhibitors, we found that these treatments reversed suppression of FOXM1 (27), suggesting that HSP70 is a specific FOXM1 inhibitor. To investigate whether FOXM1 inhibition linked to HSP70/FOXM1 interaction, we performed reciprocal coimmunoprecipitations and found that FOXM1 interacts with HSP70 (27). In addition, we found that HSP70 is able to interfere with the transcriptional activity of FOXM1 (unpublished data). Furthermore, quantitative ChIP assays revealed that HSP70 reduces up to 2-fold the ability of exogenous FOXM1 to bind to its regulatory elements, while the levels of exogenous FOXM1 were not affected (27). These data suggest that the primary effect of HSP70 on FOXM1 is the inhibition of its transactivation via binding and that the suppression of FOXM1 expression is a secondary effect.

Synergy between FOXM1 and CENPF to stimulate prostate cancer

CENPF (centromere protein F) encodes a protein that associates with the centromere–kinetochore complex (28) and COUP-TFII,

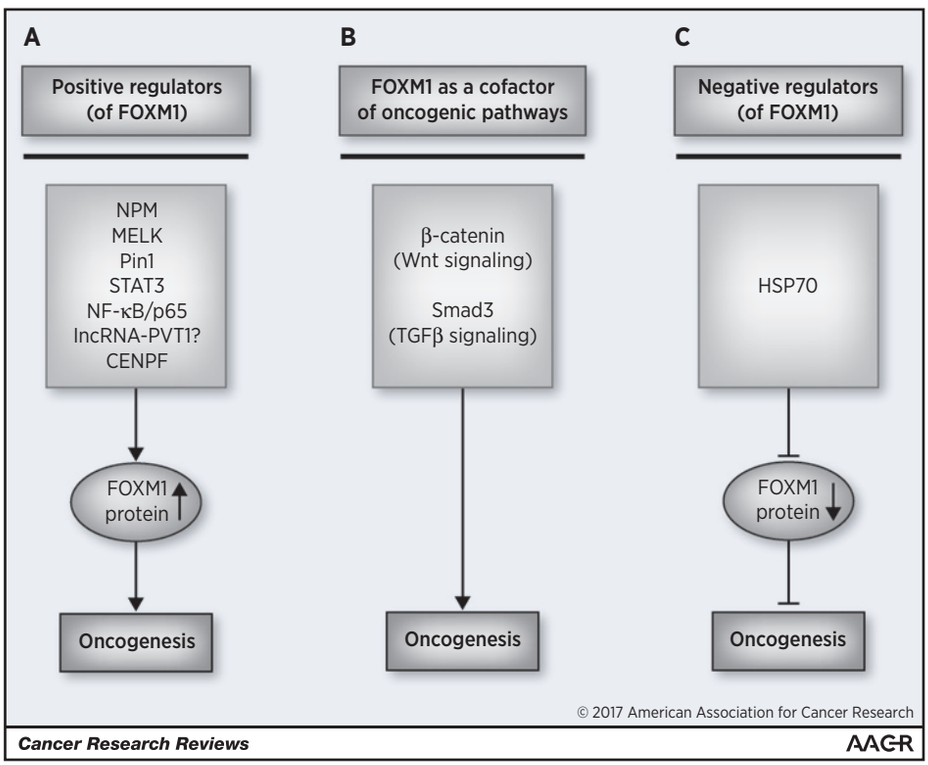

Figure 1.
Different examples of FOXM1 interactions in cancer. **A,** Several proteins stabilize FOXM1 and increase its oncogenic activity. **B,** FOXM1 as an assembly factor contributes to Wnt and TGFβ signaling. **C,** Proteotoxic stress suppresses FOXM1 via upregulation of HSP70 and its interaction with FOXM1.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/77/12/3135/2748374/3135.pdf by guest on 11 August 2024

an orphan nuclear receptor (29). The localization of CENPF points toward its role in chromosome segregation during mitosis. It turned out that simultaneous FOXM1 and CENPF overexpression in prostate cancer is linked to the loss of miRNAs, miR-101 and miR-27a. FOXM1 and CENPF are direct targets of COUP-TFII, which is targeted by miR-101 and miR-27a. Overexpression of FOXM1 and CENPF in prostate cancer is indirectly regulated by the loss of miR-101 and miR-27a as a primary event leading to activation of COUP-TFII that induces FOXM1 and CENPF (30). Computational analysis of genome-wide regulatory networks for human and mouse prostate cancer has identified FOXM1 and CENPF as synergistic master regulators of prostate cancer (28). When FOXM1 and/or CENPF was silenced individually or together in human prostate cell lines or in prostate xenograft tumors, the anticancer response was synergistic (28). Interestingly, coexpression of FOXM1 and CENPF in prostate cancer patient samples is a predictor of poor prognosis. Although FOXM1 does not directly interact with CENPF, it synergistically induces prostate cancer via upregulation of PI3K and MAPK signaling pathways (28).

Summary

The oncogenic transcription factor FOXM1 is overexpressed in the majority of human cancers and contributes to all hallmarks of cancer (3). Typically, FOXM1 induces cancer development by the transcriptional activation of its targets. Several proteins or RNAs interact with FOXM1 and stabilize FOXM1 expression and increase FOXM1 activity in cancer cells (Fig. 1A). However, it has became clear that FOXM1 may also act as a cofactor for the activation or the enhancement of other oncogenic pathways. For example, FOXM1 stabilizes β-catenin to induce the Wnt pathway (22) and SMAD3 to induce the TGFβ pathway in cancer cells (Fig. 1B; ref. 23). In addition, direct interaction between HSP70 and FOXM1 leads to suppression of FOXM1 (Fig. 1C). The information from this article will help to devise novel FOXM1 inhibitors that either will hinder FOXM1 protein–protein/RNA interactions or will mimic FOXM1-repressive proteins and will be valuable anticancer drugs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

I thank Dr. Marianna Halasi (UIC) for her valuable comments and for the preparation of the figure. I thank Dr. Irum Khan for fruitful discussions.

Grant Support

This work was supported by NIH grants 1R01CA129414, 5R01CA138409, 1R21CA134615, and 5R21CA194608.

Received December 30, 2016; revised March 9, 2017; accepted April 20, 2017; published OnlineFirst June 5, 2017.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/77/12/3135/2748374/3135.pdf by guest on 11 August 2024

References

1. Laoukili J, Stahl M, Medema RH. FoxM1: At the crossroads of ageing and cancer. Biochim Biophys Acta 2007;1775:92–102.
2. Pilarsky C, Wenzig M, Specht T, Saeger HD, Grutzmann R. Identification and validation of commonly overexpressed genes in solid tumors by comparison of microarray data. Neoplasia 2004;6:744–50.
3. Halasi M, Gartel AL. FOX(M1) News–It Is Cancer. Mol Cancer Ther 2013;12:245–54.
4. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–74.
5. Bhat UG, Jagadeeswaran R, Halasi M, Gartel AL. Nucleophosmin interacts with FOXM1 and modulates the level and localization of FOXM1 in human cancer cells. J Biol Chem 2011;286:41425–33.
6. Pandit B, Halasi M, Gartel AL. p53 negatively regulates expression of FoxM1. Cell Cycle 2009;8:3425–7.
7. Barsotti AM, Prives C. Pro-proliferative FoxM1 is a target of p53-mediated repression. Oncogene 2009;28:4295–305.
8. Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. Nat Med 2015;21:938–45.
9. Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med 2005;352:254–66.
10. Quentmeier H, Martelli MP, Dirks WG, Bolli N, Liso A, Macleod RA, et al. Cell line OCI/AML3 bears exon-12 NPM gene mutation-A and cytoplasmic expression of nucleophosmin. Leukemia 2005;19:1760–7.
11. Khan I, Halasi M, Zia MF, Gann P, Gaitonde S, Mahmud N, Gartel AL. Nuclear FOXM1 drives chemoresistance in AML. Leukemia 2017;31, 251–5.
12. Ganguly R, Hong CS, Smith LG, Kornblum HI, Nakano I. Maternal embryonic leucine zipper kinase: key kinase for stem cell phenotype in glioma and other cancers. Mol Cancer Ther 2014;13:1393–8.
13. Joshi K, Banasavadi-Siddegowda Y, Mo X, Kim SH, Mao P, Kig C, et al. MELK-dependent FOXM1 phosphorylation is essential for proliferation of glioma stem cells. Stem Cells 2013;31:1051–63.
14. Radhakrishnan SK, Bhat UG, Hughes DE, Wang IC, Costa RH, Gartel AL. Identification of a Chemical Inhibitor of the Oncogenic Transcription Factor Forkhead Box M1. Cancer Res 2006;66:9731–35.
15. Bhat UG, Halasi M, Gartel AL. FoxM1 is a general target for proteasome inhibitors. PLoS One 2009;4:e6593.
16. Kruiswijk F, Hasenfuss SC, Sivapatham R, Baar MP, Putavet D, Naipal KA, et al. Targeted inhibition of metastatic melanoma through interference with Pin1-FOXM1 signaling. Oncogene 2016;35: 2166–77.
17. Maachani UB, Shankavaram U, Kramp T, Tofilon PJ, Camphausen K, Tandle AT. FOXM1 and STAT3 interaction confers radioresistance in glioblastoma cells. Oncotarget 2016;7:77365–77.
18. Jin B, Wang C, Li J, Du X, Ding K, Pan J. Anthelmintic niclosamide disrupts the interplay of p65 and FOXM1/beta-catenin and eradicates leukemia stem cells in chronic myelogenous leukemia. Clin Cancer Res 2017;23:789–803.
19. Xu MD, Wang Y, Weng WW, Wei P, Qi P, Zhang Q, et al. A positive feedback loop of lncRNA-PVT1 and FOXM1 facilitates gastric cancer growth and invasion. Clin Cancer Res 2017;23:2071–80.
20. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell 2006;127:469–80.
21. Ma S, Tang KH, Chan YP, Lee TK, Kwan PS, Castilho A, et al. miR-130b promotes CD133(+) liver tumor-initiating cell growth and self-renewal via tumor protein 53-induced nuclear protein. Cell Stem Cell 2010;7:694–707.
22. Zhang N, Wei P, Gong A, Chiu WT, Lee HT, Colman H. FoxM1 promotes beta-catenin nuclear localization and controls Wnt target-gene expression and glioma tumorigenesis. Cancer Cell 2011;20:427–42.
23. Xue J, Lin X, Chiu WT, Chen YH, Yu G, Liu M,et al. Sustained activation of SMAD3/SMAD4 by FOXM1 promotes TGF-beta-dependent cancer metastasis. J Clin Invest 2014;124:564–79.
24. Sundqvist A, Ten Dijkevan Dam P. Key signaling nodes in mammary gland development and cancer: Smad signal integration in epithelial cell plasticity. Breast Cancer Res 2012;14:204.
25. Pan Y, Li J, Zhang Y, Wang N, Liang H, Liu Y, et al. Slug-upregulated miR-221 promotes breast cancer progression through suppressing E-cadherin expression. Sci Rep 2016;6:25798.
26. Halasi M, Gartel AL. A novel mode of FoxM1 regulation: positive autoregulatory loop. Cell Cycle 2009;8:1966–7.

27. Halasi M, Varaljai R, Benevolenskaya E, Gartel AL. A novel function of molecular chaperone HSP70: suppression of oncogenic foxm1 after proteotoxic stress. J Biol Chem 2016;291:142–8.
28. Aytes A, Mitrofanova A, Lefebvre C, Alvarez MJ, Castillo-Martin M, Zheng T, et al. Cross-species regulatory network analysis identifies a synergistic interaction between FOXM1 and CENPF that drives prostate cancer malignancy. Cancer Cell 2014;25:638–51.
29. Xu M, Qin J, Tsai SY, Tsai MJ. The role of the orphan nuclear receptor COUP-TFII in tumorigenesis. Acta Pharmacol Sin 2015;36:32–6.
30. Lin SC, Kao CY, Lee HJ, Creighton CJ, Ittmann MM, Tsai SJ, et al. Dysregulation of miRNAs-COUP-TFII-FOXM1-CENPF axis contributes to the metastasis of prostate cancer. Nat Commun 2016;7:11418.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/77/12/3135/2748374/3135.pdf by guest on 11 August 2024