Distinct Epigenetic Mechanisms Distinguish *TMPRSS2–ERG* Fusion-Positive and -Negative Prostate Cancers

Joshi J. Alumkal and **James G. Herman**
Oregon Health & Science University, Knight Cancer Institute, Portland, Oregon

Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland

Summary

This issue of *Cancer Discovery* features an article that describes distinct epigenetic mechanisms that operate in *TMPRSS2–ERG* fusion-negative prostate cancers. This finding clarifies molecular features of these *TMPRSS2–ERG* fusion-negative tumors and may have implications for how to treat this prostate cancer subtype.

Prostate cancer is the most common cancer in men in the United States and the second leading cause of cancer-related mortality in men in the United States (1). Despite screening and early detection, it is estimated that 241,740 men will be diagnosed with prostate cancer this year, and 28,170 men are predicted to die from this disease in 2012 (1). Androgens, or male hormones, remain key drivers of prostate cancer growth at all stages of this disease (2). Indeed, newer treatments that lower androgen levels or interfere with androgen activation of the androgen receptor have been developed, and these drugs extend survival (3). However, disease progression despite these agents is universal (3). Therefore, there is an urgent need to understand additional mechanisms that may promote prostate cancer development and progression to inform new therapeutic strategies.

Recent studies show that the number of genetic changes in prostate cancer is fewer than in many other malignancies (4, 5), suggesting that other processes may be driving this malignancy. Another mechanism that contributes to cancer development and progression is epigenetic change—the heritable control of gene expression in the absence of DNA sequence changes (6). Examples of epigenetic changes include histone modifications, including repressive histone methylation changes and gain and loss of DNA methylation. A key protein that links histone methylation, DNA methylation, and gene repression is the EZH2 histone methyltransferase, a polycomb protein, which is commonly upregulated in prostate cancer (7, 8). There are several potential mechanisms that play a role in EZH2 upregulation in prostate cancer with much of this involving the transcription factor ERG. First, *EZH2* is a target of the *TMPRSS2–ERG* gene fusion, and TMPRSS2–ERG and EZH2 cooperate in the regulation of shared target genes (9). *TMPRSS2–ERG* gene fusions are an early and important driver of prostate cancer development, and these gene fusions are present in approximately 50% of patients with prostate cancer (10). However, mechanisms

Corresponding Authors: James G. Herman, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Oncology, 1650 Orleans Street, Cancer Research Building-1, Room 2M44, Baltimore, MD 21231. Phone: 410-955-8506; Fax: 410-614-9884; hermaji@jhmi.edu; and Joshi J. Alumkal, Knight Cancer Institute, Oregon Health & Science University, 3303 SW Bond Avenue, MC CH14R, Portland, OR 97239. Phone: 503-494-1091; Fax: 503-494-6197; alumkalj@ohsu.edu.

Disclosure of Potential Conflicts of Interest
J.G. Herman has a commercial research grant from MDX Health and is a consultant/advisory board member of MDX Health. No potential conflicts of interest were disclosed by the other author.

responsible for the development and progression of *ERG* fusion-negative (FUS−) prostate cancer have generally not been understood.

In this issue of *Cancer Discovery*, Börno and colleagues (11) describe distinct differences in patterns of DNA methylation and specific genes involved in epigenetic regulation between *TMPRSS2–ERG* fusion-positive (FUS+) and FUS− tumors. They describe increased DNA methylation events in FUS− tumors that may underlie the development and progression of these prostate tumors. To determine the methylation differences between normal prostate samples and prostate cancer samples, the authors used a deep sequencing read-out of the MeDIP (methylated DNA immunoprecipitation) technique called MeDIP-Seq. Using 53 normal prostate samples to determine tumor-specific alterations, they examined 17 FUS+ and 20 FUS− tumors. The authors found that there were significant differences in DNA methylation between normal prostate samples and the prostate tumor samples, as expected, given previous studies of prostate tumors and other forms of cancer. However, the distinct differences between FUS+ and FUS− tumors were not expected, and the authors determined that FUS− samples had significantly more DNA methylation alterations than FUS+ samples. In fact, the FUS+ samples had overall similar levels of DNA methylation at the loci examined compared with normal prostate samples.

To understand mechanisms that might account for this increased DNA methylation in FUS− prostate cancers, the authors quantified gene expression levels of the DNA methyltransferase (DNMT) enzymes *DNMT1, DNMT3A, DNMT3B*, and the histone methyltransferase enzymes *EZH1* and *EZH2*. Although *DNMT1* and *DNMT3A* were upregulated in tumors compared with normal, an observation made for other forms of cancer, at least in the case of *DNMT1*, the level of *EZH2* was distinct. Although *EZH2* is a TMPRSS2–ERG target gene, and indeed FUS+ tumors had elevated levels of *EZH2* compared with normal prostate, *EZH2* mRNA levels were significantly higher in FUS− than FUS+ tumors. Despite the known role of the *c-Myc* oncogene in increasing *EZH2* expression, *c-Myc* levels were similar between FUS+ and FUS− cancers. Several microRNAs have been shown to regulate *EZH2* expression, leading the authors to examine expression levels of these microRNAs in normal prostate samples, FUS− cancers, and FUS+ cancers (12, 13). Notably, *miR-26a* expression was significantly lower in FUS− prostate cancers than FUS+ prostate cancers, and a direct testing of this association was provided by overexpression of a *miR-26a* mimic, which suppressed *EZH2* expression in the FUS− DU145 prostate cancer cell line with high basal levels of *EZH2*.

Because the FUS− cancers had significantly greater DNA methylation events, higher *EZH2* expression, and lower *miR-26a* expression levels than FUS− cancers, the authors next determined whether DNA methylation–induced silencing of the *miR-26a* locus might explain that effect. Hypermethylation of the *miR-26a* locus was present in FUS− prostate cancers, but not FUS+ prostate cancers, and there was a strong inverse correlation between *miR-26a* DNA methylation and *miR-26a* expression. *EZH2* expression was also inversely correlated with *miR-26a* expression, suggesting that the suppression of *miR-26a* expression by DNA methylation might contribute to EZH2 upregulation in FUS− prostate cancers. Indeed, the authors confirmed that the DNA methylation of the *miR-26a* locus was functional using 2 approaches. First, *in vitro* methylation of a construct containing the *miR-26a* locus suppressed *miR-26a* expression, whereas treatment of prostate cancer cells with the DNMT inhibitor 5-aza-2′deoxycytidine reactivated *miR-26a* expression and suppressed *EZH2* expression. These results confirm the important role of *miR-26a* DNA methylation in regulating *EZH2* expression in prostate cancer. However, they raise an interesting dilemma: Is *mir-26a* methylation and silencing a cause of increased EZH2 or is increase of EZH2 a factor leading to increased genomic DNA methylation including *mir-26a*? This integration may provide a positive loop accelerating the process of prostate

tumorigenesis irrespective of which event is the chicken or the egg. Indeed, unlike genetic events including the generation of the *TMPRSS2–ERG* fusion, which occur at a single time point, epigenetic dysregulation may be progressive.

These interesting studies provide new information about FUS⁻ prostate cancers and mechanisms that may promote their development and progression. However, several questions still need to be addressed in additional studies. Although *EZH2* levels are higher in FUS⁻ prostate cancers than FUS⁺ prostate cancers, both of these subsets of prostate cancer have higher *EZH2* levels than normal prostate samples. This reinforces previous studies, which support an important role for EZH2 dysregulation in prostate cancer (7). Because TMPRSS2–ERG has been shown to cooperate with EZH2 to regulate gene expression, is this a more efficient way to dysregulate specific targets, whereas a FUS⁻ tumor uses a less directed means to silence the genome? This may require a more detailed examination of the specific silenced loci in FUS⁺ versus FUS⁻ tumors, and specifically those, which are known *EZH2* targets. Because TMPRSS2–ERG does not direct EZH2 to chromatin, what other proteins may contribute to the higher frequency of DNA methylation events in FUS⁻ prostate cancers? Could *TMPRSS2–ERG* play a role in preventing DNA methylation in FUS⁺ prostate cancers, and if so, how? Finally, *ERG* is an EZH2 target gene and DNA methylation of the *ERG* gene is common in prostate cancer (14). Could *ERG* silencing through this mechanism lead to selective pressure for *TMPRSS2–ERG* gene fusions in some prostate tumors?

This report by Börno and colleagues (11) provides key insights into previously unrecognized epigenetic differences between FUS⁺ and FUS⁻ prostate cancers. Their results suggest that as DNA methylation occurs more frequently in FUS⁻ tumors, these changes may be more important for FUS⁻ prostate cancer development and progression. In addition, they show a key role for *miR-26a* DNA methylation, which provides a mechanism for *EZH2* upregulation in these cancers. Because DNMT inhibitors are approved for the treatment of myelodysplasia and leukemia, and at least one agent has been shown to suppress EZH2 function in cancer, therapeutic strategies that target epigenetic regulation may have different activities in FUS⁺ and FUS⁻ prostate cancer (15). We anticipate that these drugs, or other epigenetic therapies, will be tested prospectively in clinical trials in men with prostate cancer, and the success of these trials may be different according to *TMPRSS2–ERG* fusion status.

Acknowledgments

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012; 62:10–29. [PubMed: 22237781]

2. Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalhorn TF, Higano CS, et al. Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. Cancer Res. 2008; 68:4447–54. [PubMed: 18519708]

3. de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med. 2011; 364:1995–2005. [PubMed: 21612468]

4. Berger MF, Lawrence MS, Demichelis F, Drier Y, Cibulskis K, Sivachenko AY, et al. The genomic complexity of primary human prostate cancer. Nature. 2011; 470:214–20. [PubMed: 21307934]

5. Kan Z, Jaiswal BS, Stinson J, Janakiraman V, Bhatt D, Stern HM, et al. Diverse somatic mutation patterns and pathway alterations in human cancers. Nature. 2010; 466:869–73. [PubMed: 20668451]

6. Jones PA, Baylin SB. The epigenomics of cancer. Cell. 2007; 128:683–92. [PubMed: 17320506]

7. Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, et al. The polycomb group protein EZH2 is involved in progression of prostate cancer. Nature. 2002; 419:624–9. [PubMed: 12374981]

8. Viré E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, et al. The Polycomb group protein EZH2 directly controls DNA methylation. Nature. 2006; 439:871–4. [PubMed: 16357870]

9. Yu J, Yu J, Mani RS, Cao Q, Brenner CJ, Cao X, et al. An integrated network of androgen receptor, polycomb, and TMPRSS2-ERG gene fusions in prostate cancer progression. Cancer Cell. 2010; 17:443–54. [PubMed: 20478527]

10. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science. 2005; 310:644–8. [PubMed: 16254181]

11. Börno ST, Fischer A, Kerick M, Falth M, Liable M, Brase JC, et al. Genome-wide DNA methylation events in *TMPRSS2–ERG* fusion negative prostate cancers implicate an EZH2-dependent mechanism with *miR-26a* hypermethylation. Cancer Discov. 2012; 2:1024–35. [PubMed: 22930729]

12. Varambally S, Cao Q, Mani RS, Shankar S, Wang X, Ateeq B, et al. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science. 2008; 322:1695–9. [PubMed: 19008416]

13. Alajez NM, Shi W, Hui AB, Bruce J, Lenarduzzi M, Ito E, et al. Enhancer of Zeste homolog 2 (EZH2) is overexpressed in recurrent nasopharyngeal carcinoma and is regulated by miR-26a, miR-101, and miR-98. Cell Death Dis. 2010; 1:e85. [PubMed: 21368858]

14. Schwartzman J, Mongoue-Tchokote S, Gibbs A, Gao L, Corless CL, Jin J, et al. A DNA methylation microarray-based study identifies ERG as a gene commonly methylated in prostate cancer. Epigenetics. 2011; 6:1248–56. [PubMed: 21946329]

15. Tan J, Yang X, Zhuang L, Jiang X, Chen W, Lee PL, et al. Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. Genes Dev. 2007; 21:1050–63. [PubMed: 17437993]