Published in final edited form as:

Clin Cancer Res. 2019 February 15; 25(4): 1139–1141. doi:10.1158/1078-0432.CCR-18-3410.

Alternative Acts: Oncogenic Splicing of Steroidogenic Enzymes in Prostate Cancer

Elahe A. Mostaghel^{1,2}

¹Geriatric Research, Education and Clinical Center, VA Puget Sound Health Care System, Seattle, WA

²Fred Hutchinson Cancer Research Center, Seattle WA

Summary

Castration resistant prostate cancer is characterized by loss of the androgen inactivation enzyme HSD17B2, emphasizing the importance of intratumoral androgens in tumor progression. Inactive isoforms generated by alternative splicing destabilize the wild-type enzyme, adding steroidogenesis to other prostate cancer drivers that undergo oncogenic splicing, highlighting aberrant splicing as a therapeutic target.

In this issue of Clinical Cancer Research, Gao and colleagues elucidate the role of HSD17B2 in modulating tumor steroid levels in castration resistant prostate cancer (CRPC). [1] They show that CRPC is characterized by functional silencing of this enzyme, which is otherwise responsible for the reverse metabolism of the active androgens, testosterone (T) and DHT, to their inactive precursors, androstenedione (AED) and androstanedione. Silencing occurred via DNA methylation, as well as generation of two alternatively spliced, catalytic-deficient isoforms that bind wild type HSD17B2 and promote its degradation. Splicing factors SRSF1 (also known as ASF1/SF2) and SRSF5 (also known as SRp40) participated in generation of the new isoforms.

It is now well-appreciated that castration does not eliminate androgens from the prostate tumor microenvironment. Castration resistant tumors are characterized by elevated tumor androgens capable of activating androgen receptor (AR) signaling programs, and residual intratumoral androgens are implicated in nearly every mechanism by which AR-mediated signaling promotes castration resistant disease. The clinical relevance of intratumoral androgens in promoting tumor growth is confirmed by the efficacy of new ligand synthesis inhibitors such as abiraterone and potent AR inhibitors such as enzalutamide in men with either castration sensitive or castration resistant disease.

The work of Gao et al joins the body of literature demonstrating that primary prostate cancer (PCa) and castration resistant tumors are characterized by a number of steroid enzyme

Corresponding Author Elahe A. Mostaghel MD PHD, Geriatric Research, Education and Clinical Center S-182, VA Puget Sound Health Care System, 1660 South Columbian Way, Seattle, WA 98108-1597, emostagh@fredhutch.org.

Conflicts of Interest

The authors declare no conflicts of interest

alterations acting to enhance utilization of circulating adrenal androgens, inhibit metabolism of T and DHT to inactive metabolites, and in the case of CRPC tumors, promote *de novo* androgen synthesis. [2]

Differential changes in the expression of reductive and oxidative enzyme pairs favoring the conversion of inactive diones (e.g. AED and androstenedione) to active androgens (e.g. T and DHT, respectively) have been observed in both primary PCa and CRPC tumors (Fig 1A). These changes include increased tumor expression of the reductive enzymes HSD17B3 and AKR1C3, and decreased expression of the oxidative enzymes HSD17B2 and HSD17B4, suggesting a shift in tumoral androgen metabolism to the formation of T and DHT. [2, 3] Similarly, primary PCa demonstrates a selective loss of both AKR1C2 and AKR1C1 versus paired benign tissues, resulting in a reduced capacity to metabolize DHT to 3 α -diol, and thereby increasing tumoral DHT levels. Increased expression of HSD17B10 and HSD17B6 (RLHSD), oxidative enzymes capable of mediating the back conversion of 3 α -diol to DHT, has also been observed in malignant epithelial cells compared to normal, similarly consistent with an increased capacity to generate DHT in tumor tissue. [2]

In CRPC tumors the expression of numerous transcripts involved in *de novo* androgen biosynthesis and adrenal androgen utilization is altered, including increased expression of STAR, CYP17A, HSD3B1/2, HSD17B3, AKR1C3, and SRD5A, all of which promote DHT synthesis. A gain of function SNP in HSD3B1 (1245C>N367T) has recently been identified in CRPC, which renders the protein resistant to ubiquitination and degradation, resulting in increased protein levels of HSD3B1 and increased levels of intratumoral DHT production. [2] These observations suggest that tissue-based alterations in steroid metabolism contribute to the development of CRPC and underscore these metabolic pathways as critical targets of therapy.

The current study by Gao et al, along with the recent work of Ko et al which demonstrated a similar loss of the androgen inactivating isoform of HSD17B4 in CRPC, highlights the potentially equal important role of androgen inactivation vs androgen synthesis in maintaining CRPC tumor androgen levels. [3] While clinical efforts have focused on inhibition of ligand synthesis, the specific loss of androgen inactivating enzymes such as HSD17B2 and HSD17B4 demonstrates that CRPC tumors harbor pre-existing mechanisms that, by conserving residual androgens in their active form, will amplify the effect of even low androgen levels in driving ligand-mediated AR signaling, contributing to development of resistant disease. It remains to be determined whether loss of HSD17B2 or HSD17B4 is associated with higher androgen levels in CRPC tumors, and whether these tumors are therefore more likely to respond to continued AR and ligand synthesis directed therapy vs tumors that are driven by ligand-independent mechanisms.

The silencing of functional enzyme expression for both HSD17B2 and HSD17B4 occurred via alternative splicing. Gao et al identified two catalytic-deficient isoforms of HSD17B2 and showed that binding to these isoforms destabilized wild type HSD17B2 and promoted its degradation. Ko et al demonstrated that of five splice forms of HSD17B4, only isoform 2 was capable of metabolizing T and DHT and that expression of this isoform was specifically lost in CRPC.[3] While the factors involved in alternative splicing of HSD17B4 were not

investigated, Gao et al found that overexpression of SRSF1 or SRSF5 resulted in generation of the truncated HSD17B2 isoforms.

Notably, SRSF1 and SRSF5 are involved in the oncogenic splicing of multiple genes implicated in driving PCa progression, including BCL-X, CCND1, KLF6, VEGF, and perhaps of most interest, AR (Fig 1B).[4, 5] Splice variants of the AR which lack the C terminal ligand binding domain (LBD) but retain the DNA binding and N terminal domains required for AR dimerization, DNA binding and transcriptional regulation have been described. Among these, the ligand independent, constitutionally active AR variant 7 (AR-V7) is the most common variant in CRPC and has been shown to be an adverse prognostic and predictive marker. The splicing factors U2AF55 and SRSF1 were shown to act as pioneer factors, specifically recruiting the spliceosome to the 3' splice site of AR-V7, thus increasing the expression of AR-V7 mRNA.[4] Thus, the novel observation that SRSF1 and SRSF5 are involved in the alternative splicing of HSD17B2 adds steroidogenic enzymes to the known suite of drivers regulated by these splicing factors, and suggests the adverse outcomes associated with each of these genes in PCa may actually reflect the combined activity of multiple tumor promoting changes mediated by the activity of a common splicing factor.

From a therapeutic standpoint, while evaluation of DNA methyltransferase inhibitors to induce re-expression of HSD17B2 is worth consideration, the splicing data nominate SRSF1 as a multifunctional target whose inhibition could have anti-tumor efficacy via multiple, mechanistically unrelated pathways. Thus, rather than approaches such as splice-switching oligonucleotides (SSOs) or small interference RNA (siRNA) that target individual gene-specific splicing events, strategies that target SRSF1 itself may be more effective. While clinical efforts to date are limited, the BET inhibitor JQ1 decreased expression of AR-V7 in preclinical models of CRPC by downregulating activity of the splicing factors SRSF1 and U2AF65.[4] Likewise, small molecular inhibitors of SRPK1, the splice factor kinase that phosphorylates and activates SRSF1, are already in pre-clinical development in PCa models of angiogenesis and would appear to warrant testing in more diverse PCa tumor models.[6]

In summary, the work by Gao et al elucidates the contribution of HSD17B2 to steroidogenesis, further emphasizing the importance of intratumoral steroid levels in CRPC tumors and highlights the potential prognostic and therapeutic implications when multiple tumor promoting changes are mediated by the activity of a common splicing factor.

Acknowledgments:

E. A. Mostaghel is funded by NIH Pacific Northwest Prostate Cancer SPORE P50 CA97186, NIH P01 CA163227, DOD W81XWH-12-1-0208, and Department of Veterans Affairs Puget Sound Health Care System.

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Figure 1. Induction of growth promoting vs growth inhibiting factors in prostate cancer (PCa).

A) Pathways of androgen biosynthesis. Cholesterol is converted to pregnenolone by the action of STAR and CYP11A, and then to the adrenal androgen DHEA by the sequential hydroxylase and lyase activity of CYP17A. DHEA (from *de novo* or circulating sources) is acted on by HSD3B to form androstenedione (AED), then SRD5A to form androstenedione, and AKR1C2 to form androsterone. Each of these steroids can be acted on in parallel by the reductive activity of AKR1C3 or HSD17B3 to generate A5-diol, testosterone (T), DHT or 3α-diol, respectively, and this reaction can be reversed by the activity of HSD17B2 or HD17B4. 3α-diol can undergo glucuronidation by UGT2b15 or UGT2B17 for excretion, or can be metabolized back to DHT by HSD17B6 (RLHSD) or HSD17B10. Enzymes within pink arrows lead to production of steroids that are active in binding the androgen receptor (AR, in red); enzymes in green arrows lead to production of steroids with minimal capacity for AR activation (in green). **B) Genes known to undergo oncogenic splicing by SRSF1 and/or SRSF5 in prostate.** Cyclin D1 (CCND1), a key regulator of cell cycle, expresses two isoforms with opposing functions. While the canonical cyclin D1a isoform represses the transcriptional activity of AR, the truncated D1b isoform is up-regulated in PCa by the activity of SRSF1 promotes metastasis. Kruppel-like factor 6 (KLF6) regulates cell proliferation through induction of the cell cycle inhibitor p21. In PCa, a single nucleotide substitution in intron 1 creates a novel binding site for the splicing factor SRSF5, which generates a truncated splice variant-1 (SV1) that functionally antagonizes activity of the full length KLF6, thereby preventing upregulation of p21. The BCL-X gene regulates apoptosis and also encodes two isoforms with opposing functions. The pro-apoptotic short isoform, BCL-XS, is down-regulated in many cancers including PCa, while SRSF1 enhances splicing and upregulation of the anti-apoptotic long isoform, BCL-XL. Vascular endothelial growth factor (VEGF), a key driver of angiogenesis, encodes multiple isoforms, including two with antagonistic properties. The VEGF165b isoform, downregulated in PCa, cannot signal through VEGFR2 and inhibits angiogenesis, whereas the VEGF165a isoform, generated by the activity of SRSF1 and SRSF5, binds and signals via VEGFR2 to promote angiogenesis. AR can be spliced to multiple constitutively active isoforms lacking the ligand binding

domain. SRSF1 promotes splicing of full length AR to the most clinically relevant isoform, the constitutively active AR variant 7 (AR-V7). The long (L) form of the steroidogenic enzyme HSD17B2 catalyzes inactivation of active steroids. SRSF1 and SRSF5 promotes generation of the catalytic-deficient medium (M) and short (S) isoforms, which bind and destabilize HSD17B2-L promoting its degradation. Growth promoting vs growth inhibitory isoforms are shown in red vs green, respectively. Where known, relevant splicing factors besides SRSF1 and SRSF5 (in bold font) are also indicated (blue circles).