Clinical Cancer Research

Alternative Acts: Oncogenic Splicing of Steroidogenic Enzymes in Prostate Cancer

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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.

See related article by Gao et al., p. 1291

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; ref. 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 and colleagues joins the body of literature demonstrating that primary prostate cancer and castration-resistant tumors are characterized by a number of steroid enzyme 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 androstanedione) to active androgens (e.g., T and DHT, respectively) have been observed in both primary prostate cancer 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 prostate cancer demonstrates a selective loss of both AKR1C2 and AKR1C1 versus paired benign tissues, resulting in a reduced capacity to metabolize DHT to 3α-diol, and thereby increasing tumoral DHT levels. Increased expression of HSD17B10 and HSD17B6 (RLHSD), oxidative enzymes capable of mediating the back conversion of 3α-diol to DHT, has also been observed in malignant epithelial cells compared with 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.

This study by Gao and colleagues, along with the recent work of Ko and colleagues which demonstrated a similar loss of the androgen-inactivating isoform of HSD17B4 in CRPC, highlights the potentially equal important role of androgen inactivation versus androgen synthesis in maintaining CRPC tumor androgen levels (3). Although 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 preexisting 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 versus tumors that are driven by ligand-independent mechanisms.

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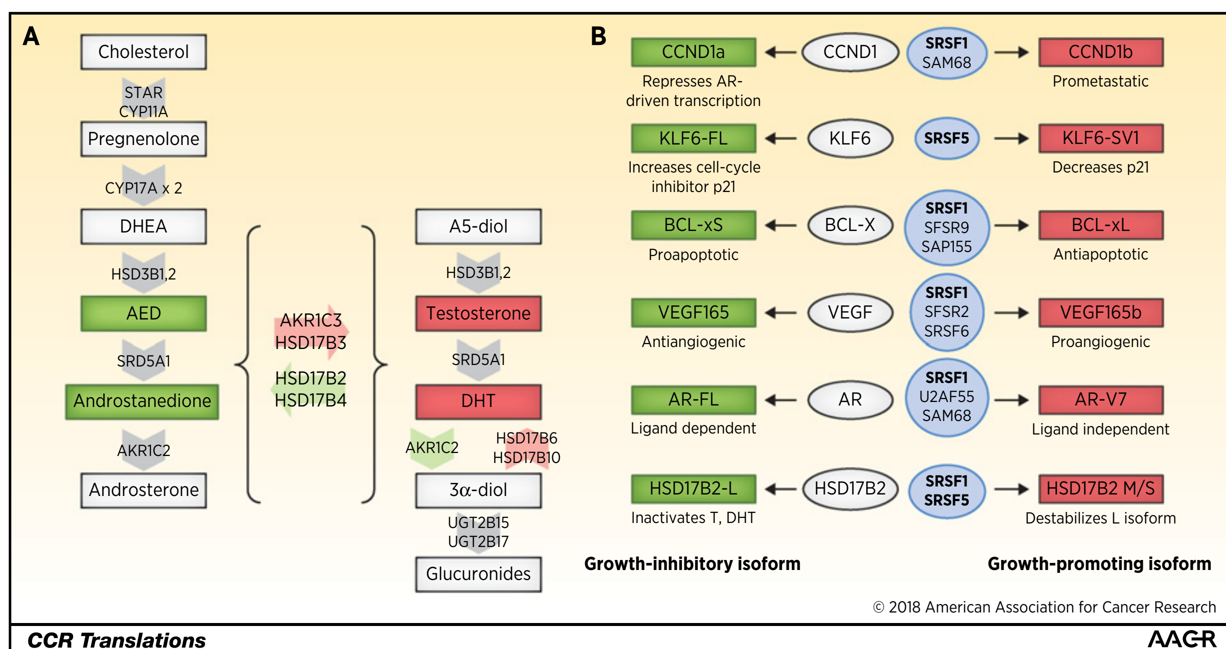

Figure 1.
Induction of growth-promoting versus growth-inhibiting factors in prostate cancer. **A,** Pathways of androgen biosynthesis. Cholesterol is converted to pregnenolone by the action of STAR and CYP11A, and then to the adrenal androgen DHEA by the sequential hydroxylase and lyase activity of CYP17A. DHEA (from *de novo* or circulating sources) is acted on by HSD3B to form androstenedione (AED), then SRD5A to form androstanedione, 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 glucuronidaton 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 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, a key regulator of the cell cycle, expresses two isoforms with opposing functions. Although the canonical cyclin D1a isoform represses the transcriptional activity of AR, the truncated D1b isoform is upregulated in prostate cancer by the activity of SRSF1, promoting metastasis. Kruppel-like factor 6 (KLF6) regulates cell proliferation through induction of the cell-cycle inhibitor p21. In prostate cancer, 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 proapoptotic short isoform, BCL-xS, is downregulated in many cancers including prostate cancer, whereas SRSF1 enhances splicing and upregulation of the antiapoptotic long isoform, BCL-xL. VEGF, a key driver of angiogenesis, encodes multiple isoforms, including two with antagonistic properties. The VEGF165b isoform, downregulated in prostate cancer, cannot signal through VEGFR2 and inhibits angiogenesis, whereas the VEGF165a isoform, generated by the activity of SRSF1 and SRSF5, binds and signals via VEFGR2 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 promote generation of the catalytic-deficient medium (M) and short (S) isoforms, which bind and destabilize HSD17B2-L, promoting its degradation. Growth-promoting versus growth-inhibitory isoforms are shown in red versus green, respectively. Where known, relevant splicing factors besides SRSF1 and SRSF5 (in bold font) are also indicated (blue circles).

The silencing of functional enzyme expression for both HSD17B2 and HSD17B4 occurred via alternative splicing. Gao and colleagues identified two catalytic-deficient isoforms of HSD17B2 and showed that binding to these isoforms destablized wild-type HSD17B2 and promoted its degradation. Ko and colleagues 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). Although the factors involved in alternative splicing of HSD17B4 were not investigated, Gao and colleagues 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 prostate cancer progression, including BCL-X, CCND1, KLF6, VEGF, and perhaps of most interest, AR (Fig. 1B; refs. 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 previously. 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

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associated with each of these genes n prostate cancer may actually reflect the combined activity of multiple tumor-promoting changes mediated by the activity of a common splicing factor.

From a therapeutic standpoint, although evaluation of DNA methyltransferase inhibitors to induce reexpression of HSD17B2 is worth consideration, the splicing data nominate SRSF1 as a multifunctional target whose inhibition could have antitumor efficacy via multiple, mechanistically unrelated pathways. Thus, rather than approaches such as splice-switching oligonucleotides or siRNA that target individual gene-specific splicing events, strategies that target SRSF1 itself may be more effective. Although 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-molecule inhibitors of SRPK1, the splice factor kinase that phosphorylates and activates SRSF1, are already in preclinical development in prostate cancer models of angiogenesis and would appear to warrant testing in more diverse prostate cancer tumor models (6).

In summary, the work by Gao and colleagues 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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Gao X, Dai C, Huang S, Tang J, Chen G, Li J, et al. Functional silencing of HSD17B2 in prostate cancer promotes disease progression. Clin Cancer Res 2019;25:1291–301.
2. Mostaghel EA. Steroid hormone synthetic pathways in prostate cancer. Transl Androl Urol 2013;2:212–27.
3. Ko HK, Berk M, Chung YM, Willard B, Bareja R, Rubin M, et al. Loss of an androgen-inactivating and isoform-specific HSD17B4 splice form enables emergence of castration-resistant prostate cancer. Cell Rep 2018;22:809–19.
4. Paschalis A, Sharp A, Welti JC, Neeb A, Raj GV, Luo J, et al. Alternative splicing in prostate cancer. Nat Rev Clin Oncol 2018;15: 663–75.
5. Sette C. Alternative splicing programs in prostate cancer. Int J Cell Biol 2013;2013:458727.
6. Antonopoulou E, Ladomery M. Targeting splicing in prostate cancer. Int J Mol Sci 2018;19:1287.

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