Published in final edited form as:
J Investig Med. 2012 February ; 60(2): . doi:10.231/JIM.0b013e31823874a4.

The 5α-androstanedione pathway to dihydrotestosterone in castration-resistant prostate cancer

Nima Sharifi
Division of Hematology/Oncology, Department of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-8852, Phone: 214 645-5921, FAX: 214 645-5915, nima.sharifi@utsouthwestern.edu

Abstract

The survival and progression of prostate cancer is generally dependent on expression of the androgen receptor (AR), as well as the availability of endogenous AR agonists. Originating from the gonads, testosterone is released into circulation and is converted by steroid-5α-reductase (SRD5A) in prostate cancer to 5α-dihydrotestosterone (DHT), potently activating AR and driving tumor progression. Advanced prostate cancer is initially treated with gonadal testosterone depletion, which suppresses this cascade of events and typically leads to a treatment response. Eventually, resistance to testosterone deprivation occurs with "castration-resistant" prostate cancer (CRPC) and is driven by the intratumoral synthesis of DHT. The generation of DHT occurs in large part from adrenal 19-carbon precursor steroids, which are dependent on expression of CYP17A1. Although the path from adrenal precursor steroids to DHT was generally thought to require 5α-reduction of testosterone, recent data suggest that it instead involves conversion from Δ^4-androstenedione by SRD5A isoenzyme-1 to 5α-androstanedione, followed by subsequent conversion to DHT. The 5α-androstanedione pathway to DHT therefore bypasses testosterone entirely. Abiraterone acetate effectively inhibits CYP17A1, blocks the synthesis of androgens and extends the survival of men with CRPC. Further progress in the hormonal treatment of CRPC is dependent on an understanding of the mechanisms that underlie CRPC and resistance to abiraterone acetate.

Keywords

prostate cancer; androgens; 5α-dihydrotestosterone; castration-resistance; 5-alpha-androstanedione; testosterone; androgen receptor; 5-alpha-reductase

---

The androgen axis is central to the progression and treatment of prostate cancer. Essential components of this axis require the expression of the androgen receptor (AR) and the generation of endogenous AR agonists. The androgen signaling pathway is intimately involved from tumor initiation and invasion, to the development of metastatic disease. Translocation of the androgen-controlled TMPRSS2 regulatory region proximal to a member of the ETS-family oncogenes occurs in the transition between high grade prostatic intraepithelial neoplasia and invasive prostate cancer, driving oncogene expression[1]. The requirement for expression of these oncogenes, elicited by the androgen axis, continues to very late and resistant states of disease[2]. Therefore, the mechanisms that regulate the androgen axis, from the generation of ligand, to AR expression, to the response of AR-regulated genes, all represent steps that are potential points of intervention for the development of new pharmacologic therapies. A precise understanding of this pathway is required for further advances in the treatment of prostate cancer.

Gonadal testosterone deprivation

Physiologic serum concentrations of total testosterone (T) are generally > 300 ng/dl (10.4 nmol/l)[3]. In prostatic tissue, T is converted by steroid-5α-reductase (SRD5A) to 5α-dihydrotestosterone (DHT). T is capable of binding AR in the absence of metabolism to DHT but the latter is several fold more potent and is the major androgen bound to AR in the prostate cell nucleus[4,5]. Although two isoenzymes exist, in the prostate expression of SRD5A2 is greater than that of SRD5A1[6]. In prostatic tissue, SRD5A enzymatic activity results in DHT concentrations that are several fold higher than T and this ratio is reversed upon treatment with pharmacologic blockade of SRD5A[7,8]. The effect of gonadal testosterone deprivation is therefore likely due in large part to the depletion of intratumoral DHT. However, despite 94% reductions in serum T with medical castration, intraprostatic T and DHT are reduced by only 70 and 80%, respectively[9]. The apparent availability of precursors for the synthesis of residual intraprostatic androgens with medical castration provides a clue as to the mechanisms of resistance to depletion of gonadal T[10]. Nonetheless, responses to gonadal T depletion therapy occur in the majority of cases, although the response in the metastatic setting is nearly always temporary[11].

Castration-resistant prostate cancer

Disease that progresses in the presence of gonadal T depletion is termed “castration-resistant” prostate cancer (CRPC). Multiple lines of evidence suggest that the switch from hormone-responsive to CRPC is regulated by a gain-of-function in AR[12-14]. A multitude of mechanisms have been implicated in increasing AR-driven transcription. These vary from alterations in coactivator/corepressor expression, ligand-independent function or ligand-sensitization through growth factors or their receptors, post-translational modification of AR, increased AR expression, AR mutations that broaden the specificity for ligand, and intratumoral steroidogenesis that increases the availability of T and/or DHT [15-22]. All of these factors may contribute to some extent to the development of CRPC in a manner that is probably highly dependent on the molecular pathogenesis of individual tumors. However, the finding of biologically significant concentrations of intratumoral androgens common to the majority of tumors, coupled with clinical responses to depletion of these androgens, implicates intratumoral steroidogenesis as a major and frequent driver of CRPC[21-24].

Essential components of intratumoral steroidogenesis

The synthesis of all steroids originates from cholesterol[25]. The structural features of the initial substrate and the final product(s) must be considered in the pathway(s) from cholesterol to T and/or DHT. Cholesterol has a 27-carbon, 3β-hydroxyl, Δ^5-structure (double bond between carbons 5 and 6). Eventual conversion to 19-carbon T and/or DHT necessitates the departure of 8 carbons through 2 enzymes, 3β-hydroxyl oxidation to 3-keto, Δ^5 isomerization to Δ^4, and 17-keto reduction to a 17β-hydroxysteroid. In the adrenal, P450scc cleaves cholesterol to 21-carbon pregnenolone, which is then a substrate for CYP17A1 hydroxylase and 17, 20-lyase activity, yielding 19-carbon dehydroepiandrosterone (DHEA). DHEA and its sulfate are the major androgen precursor steroids in serum and the probable major source(s) of intratumoral androgens in CRPC[26,27]. In CRPC, DHEA is converted to Δ^4-androstenedione (AD) by 3β-hydroxysteroid dehydrogenase/isomerase (3βHSD), which is encoded by two isoenzymes[28]. 3βHSD1 is generally thought to be expressed in peripheral tissues and 3βHSD2 the responsible enzyme in steroidogenic organs[29,30]. However, expression of transcripts encoding both isoenzymes has been detected in CRPC tissues[21,31].

It generally had been assumed that the next step in the CRPC pathway is conversion of AD to T[12,32]. The presumptive conversion of AD to T was implied in part from the observations

that intratumoral T concentrations and expression of AKR1C3, which is capable of converting AD to T, are both increased in CRPC[21,33]. Expression of SRD5A1 is increased in CRPC and was generally thought to be required for the conversion from T to DHT[21,32-34].

An alternative possibility to synthesis from adrenal precursor steroids is *de novo* androgen synthesis from cholesterol, taking place entirely in CRPC tissue. This has been reported in CRPC cell lines[35]. However, the abundance of adrenal precursors in serum, the requirement for only 2-3 enzymes for the conversion from DHEA to T and DHT, and comparisons of flux through both pathways, together suggest that the adrenals are the main source for intratumoral androgens in CRPC[27].

Abiraterone acetate

CYP17A1 enzymatic activity is required for the conversion of 21-carbon steroids to 19-carbon androgens, no matter the relative contribution of the adrenal vs. *de novo* pathways to intratumoral T and DHT. Abiraterone acetate potently blocks both CYP17A1 hydroxylase and 17, 20-lyase activity[36]. Initial clinical studies of abiraterone acetate demonstrated declines in serum T and AD concentrations; however, pituitary compensation by luteinizing hormone hypersecretion, resulted in some gonadal testosterone recovery in eugonadal males[37]. In phase I/II trials in men with CRPC, PSA declines greater than 50% occurred in approximately two-thirds of patients who had not been previously treated with chemotherapy[23,38]. Pretreatment concentrations of DHEA, DHEA-S and AD in serum were associated with treatment response[23]. In a phase III trial of abiraterone acetate plus prednisone versus placebo plus prednisone in CRPC patients previously treated with docetaxel, overall survival was 3.9 months longer in the abiraterone acetate-prednisone group[39]. Progression-free survival, PSA response rate and time to PSA progression were all in favor of the abiraterone acetate-prednisone group. On the basis of these data, abiraterone acetate was approved by the United States Food and Drug Administration in April 2011 for the treatment of metastatic CRPC in men previously treated with docetaxel. Notably, progression-free survival in the abiraterone acetate-prednisone arm was 5.6 months, raising the issue of treatment options in resistant tumors. Abiraterone acetate is administered orally, is generally well-tolerated and clinically active, all suggesting that widespread use of this drug will lead to a large population of men with abiraterone acetate-resistant CRPC. Therefore, this is an urgent area of investigation. Although early preclinical data in mouse xenograft models suggest that sustained steroidogenesis is in part responsible, there is very little insight into the mechanisms that may permit androgen synthesis in clinical tumors under abiraterone acetate treatment conditions[40].

The 5α-androstanedione pathway to DHT

Defining potential points of intervention in abiraterone acetate resistant tumors must be preceded by a firm understanding of the mechanisms underlying abiraterone acetate- and castration-resistance. Increased concentrations of T and overexpression of AKR1C3 in clinical CRPC appear to support the notion that AD is converted to T, which is the immediate precursor to DHT[32]. However, AD is also a 3-keto, Δ^4-steroid, similar to T, making it a substrate for SRD5A1 that is possibly even better than T[41,42]. An alternative possibility to synthesis through T is that 5α-reduction of AD results in synthesis of 5α-androstanedione (5α-dione), which may be 17-keto reduced to DHT (Figure 1). We have recently shown that AD is preferentially 5α-reduced to 5α-dione, rather than 17-keto reduced to T, in multiple models of CRPC, as well as freshly biopsied tissue from 2 patients with metastatic CRPC[43]. Any *T* that is synthesized from AD is actually a *poorer* substrate for SRD5A compared to AD. Therefore, the preferred route from adrenal precursors to DHT is AD → 5α-dione → DHT (5α-dione pathway), rather than AD → T → DHT (conventional

pathway). Furthermore, silencing the expression of SRD5A1 blocks the conversion of AD to 5α-dione and the eventual synthesis of DHT in CRPC[43]. This suggests that the SRD5A1 up-regulation described in multiple clinical studies of CRPC tissue, serves to increase flux from AD → 5α-dione, rather than T → DHT, as previously assumed[32]. The increase in expression of AKR1C3, which reduces 17-keto to 17-hydroxysteroids, may serve to convert 5α-dione → DHT, rather than AD → T[44]. These unanticipated findings on the origins of DHT in CRPC suggest that there should be a reevaluation of current strategies of assessing response and resistance to various hormonal therapies, including abiraterone acetate, as well as a reconsideration of the consequences of potential points of pharmacologic intervention.

Clinical implications of the 5α-androstanedione pathway

An understanding of the relevant and required intratumoral intermediates en route to DHT is necessary in order to accurately characterize androgen depletion downstream of abiraterone acetate. Intratumoral T is probably not the best marker of response or resistance to abiraterone acetate, given that this is not the major DHT precursor[45]. The spectrum of intermediate metabolites as markers of response or resistance should be expanded to include 5α-dione and probably other 5α-reduced androgens, particularly given that several 5α-reduced androgens are reversibly interconvertible to DHT[27].

Intratumoral synthesis of DHT through the 5α-dione, rather than the conventional pathway via T, alters the consequences of current and potential pathway inhibitors. Trials of dual SRD5A inhibitors in CRPC only demonstrated very modest clinical activity[46,47]. This might be interpreted to indicate that DHT is unimportant in driving CRPC. Alternatively, the effects of blocking the 5α-dione pathway through genetically or pharmacologically inhibiting SRD5A1, results in diverting AD instead to increased synthesis of T[43]. The diverted pathway resulting in increased intratumoral concentrations of T probably substitutes in part for the inhibition of DHT synthesis, despite the more modest AR agonist activity of the former androgen. One possible solution to this pitfall of SRD5A inhibition is the move one step upstream in the pathway of DHT synthesis. Pharmacologic inhibition of 3βHSD blocks the conversion of DHEA to AD, AR nuclear translocation, expression of AR-responsive genes and cell growth[28]. Similar to diversion of AD by 17-keto reduction to T with SRD5A inhibition, it is possible that DHEA is also diverted by 17-keto reduction to Δ^5-androstenediol with 3βHSD inhibition. However, just as with DHEA, Δ^5-androstenediol must also be metabolized by 3βHSD in order to induce the AR-response, for both wild-type and the LNCaP mutant AR[28]. Several pharmacologic inhibitors of 3βHSD exist but they all have problems, such as partial AR agonism, that make them untenable for use in the treatment of CRPC[13].

Conclusion

The presence of intratumoral DHT in CRPC was first noted over 30 years ago. The survival benefit conferred by treatment with abiraterone acetate is the clearest evidence yet that intratumoral androgens are a main driver of the development of resistance to hormonal therapy and progression with CRPC. Although the pathway from adrenal precursor steroids to intratumoral synthesis of DHT was widely believed to require T, the main route instead circumvents T through the 5α-dione pathway and requires expression of SRD5A1. Strategies for the development of better therapeutic approaches should account for the unanticipated dominance of the 5α-dione pathway in CRPC.

Acknowledgments

This publication has been funded in part by a Howard Hughes Medical Institute Physician-Scientist Early Career Award, a Prostate Cancer Foundation Award and from grant number PC080193 from the U.S. Army Medical Research and Materiel Command.

References

1. Tomlins SA, et al. Integrative molecular concept modeling of prostate cancer progression. Nat Genet. 2007; 39:41–51. [PubMed: 17173048]
2. Attard G, et al. Characterization of ERG, AR and PTEN gene status in circulating tumor cells from patients with castration-resistant prostate cancer. Cancer Res. 2009; 69:2912–2918. [PubMed: 19339269]
3. Sharifi N, Gulley JL, Dahut WL. An update on androgen deprivation therapy for prostate cancer. Endocr Relat Cancer. 2010; 17:R305–315. [PubMed: 20861285]
4. Deslypere JP, Young M, Wilson JD, McPhaul MJ. Testosterone and 5 alpha-dihydrotestosterone interact differently with the androgen receptor to enhance transcription of the MMTV-CAT reporter gene. Mol Cell Endocrinol. 1992; 88:15–22. [PubMed: 1334007]
5. Bruchovsky N, Wilson JD. The intranuclear binding of testosterone and 5-alpha-androstan-17-beta-ol-3-one by rat prostate. J Biol Chem. 1968; 243:5953–5960. [PubMed: 5696629]
6. Russell DW, Wilson JD. Steroid 5 alpha-reductase: two genes/two enzymes. Annu Rev Biochem. 1994; 63:25–61. [PubMed: 7979239]
7. McConnell JD, et al. Finasteride, an inhibitor of 5 alpha-reductase, suppresses prostatic dihydrotestosterone in men with benign prostatic hyperplasia. J Clin Endocrinol Metab. 1992; 74:505–508. [PubMed: 1371291]
8. Mostaghel EA, et al. Variability in the androgen response of prostate epithelium to 5alpha-reductase inhibition: implications for prostate cancer chemoprevention. Cancer Res. 2010; 70:1286–1295. [PubMed: 20124490]
9. Page ST, et al. Persistent intraprostatic androgen concentrations after medical castration in healthy men. J Clin Endocrinol Metab. 2006; 91:3850–3856. [PubMed: 16882745]
10. Labrie F. Blockade of testicular and adrenal androgens in prostate cancer treatment. Nat Rev Urol. 2011; 8:73–85. [PubMed: 21243019]
11. Sharifi N, Gulley JL, Dahut WL. Androgen deprivation therapy for prostate cancer. JAMA. 2005; 294:238–244. [PubMed: 16014598]
12. Scher HI, Sawyers CL. Biology of progressive, castration-resistant prostate cancer: directed therapies targeting the androgen-receptor signaling axis. J Clin Oncol. 2005; 23:8253–8261. [PubMed: 16278481]
13. Sharifi N. New agents and strategies for the hormonal treatment of castration-resistant prostate cancer. Expert Opin Investig Drugs. 2010; 19:837–846.
14. McPhaul MJ. Mechanisms of prostate cancer progression to androgen independence. Best Pract Res Clin Endocrinol Metab. 2008; 22:373–388. [PubMed: 18471793]
15. Ponguta LA, Gregory CW, French FS, Wilson EM. Site specific androgen receptor serine phosphorylation linked to epidermal growth factor dependent growth of castration-recurrent prostate cancer. J Biol Chem. 2008
16. Mellinghoff IK, et al. HER2/neu kinase-dependent modulation of androgen receptor function through effects on DNA binding and stability. Cancer Cell. 2004; 6:517–527. [PubMed: 15542435]
17. Heinlein CA, Chang C. Androgen receptor (AR) coregulators: an overview. Endocr Rev. 2002; 23:175–200. [PubMed: 11943742]
18. Gioeli D, Paschal BM. Post-translational modification of the androgen receptor. Mol Cell Endocrinol. 2011
19. Chen CD, et al. Molecular determinants of resistance to antiandrogen therapy. Nat Med. 2004; 10:33–39. [PubMed: 14702632]

20. Gelmann EP. Molecular biology of the androgen receptor. J Clin Oncol. 2002; 20:3001–3015. [PubMed: 12089231]
21. Montgomery RB, et al. Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. Cancer Res. 2008; 68:4447–4454. [PubMed: 18519708]
22. Titus MA, Schell MJ, Lih FB, Tomer KB, Mohler JL. Testosterone and dihydrotestosterone tissue levels in recurrent prostate cancer. Clin Cancer Res. 2005; 11:4653–4657. [PubMed: 16000557]
23. Attard G, et al. Selective inhibition of CYP17 with abiraterone acetate is highly active in the treatment of castration-resistant prostate cancer. J Clin Oncol. 2009; 27:3742–3748. [PubMed: 19470933]
24. Geller J, et al. DHT concentrations in human prostate cancer tissue. J Clin Endocrinol Metab. 1978; 46:440–444. [PubMed: 87401]
25. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr Rev. 2011; 32:81–151. [PubMed: 21051590]
26. Ghayee HK, Auchus RJ. Basic concepts and recent developments in human steroid hormone biosynthesis. Rev Endocr Metab Disord. 2007; 8:289–300. [PubMed: 17926129]
27. Sharifi N, McPhaul MJ, Auchus RJ. “Getting from here to there”-mechanisms and limitations to the activation of the androgen receptor in castration-resistant prostate cancer. J Investig Med. 2010; 58:938–944.
28. Evaul K, Li R, Papari-Zareei M, Auchus RJ, Sharifi N. 3beta-hydroxysteroid dehydrogenase is a possible pharmacological target in the treatment of castration-resistant prostate cancer. Endocrinology. 2010; 151:3514–3520. [PubMed: 20534728]
29. Simard J, et al. Molecular biology of the 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase gene family. Endocr Rev. 2005; 26:525–582. [PubMed: 15632317]
30. Lorence MC, Murry BA, Trant JM, Mason JI. Human 3 beta-hydroxysteroid dehydrogenase/delta 5----4isomerase from placenta: expression in nonsteroidogenic cells of a protein that catalyzes the dehydrogenation/isomerization of C21 and C19 steroids. Endocrinology. 1990; 126:2493–2498. [PubMed: 2139411]
31. Cai C, Wang H, Xu Y, Chen S, Balk SP. Reactivation of androgen receptor-regulated TMPRSS2:ERG gene expression in castration-resistant prostate cancer. Cancer Res. 2009; 69:6027–6032. [PubMed: 19584279]
32. Ryan CJ, Tindall DJ. Androgen Receptor Rediscovered: The New Biology and Targeting the Androgen Receptor Therapeutically. J Clin Oncol. 2011
33. Stanbrough M, et al. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res. 2006; 66:2815–2825. [PubMed: 16510604]
34. Titus MA, et al. Steroid 5alpha-reductase isozymes I and II in recurrent prostate cancer. Clin Cancer Res. 2005; 11:4365–4371. [PubMed: 15958619]
35. Locke JA, et al. Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. Cancer Res. 2008; 68:6407–6415. [PubMed: 18676866]
36. Potter GA, Barrie SE, Jarman M, Rowlands MG. Novel steroidal inhibitors of human cytochrome P45017 alpha (17 alpha-hydroxylase-C17,20-lyase): potential agents for the treatment of prostatic cancer. J Med Chem. 1995; 38:2463–2471. [PubMed: 7608911]
37. O’Donnell A, et al. Hormonal impact of the 17alpha-hydroxylase/C(17,20)-lyase inhibitor abiraterone acetate (CB7630) in patients with prostate cancer. Br J Cancer. 2004; 90:2317–2325. [PubMed: 15150570]
38. Attard G, et al. Phase I clinical trial of a selective inhibitor of CYP17, abiraterone acetate, confirms that castration-resistant prostate cancer commonly remains hormone driven. J Clin Oncol. 2008; 26:4563–4571. [PubMed: 18645193]
39. de Bono JS, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med. 2011; 364:1995–2005. [PubMed: 21612468]
40. Mostaghel EA, et al. Resistance to CYP17A1 inhibition with abiraterone in castration resistant prostate cancer: Induction of steroidogenesis and androgen receptor splice variants. Clin Cancer Res. 2011

41. Thigpen AE, Cala KM, Russell DW. Characterization of Chinese hamster ovary cell lines expressing human steroid 5 alpha-reductase isozymes. J Biol Chem. 1993; 268:17404–17412. [PubMed: 8394341]

42. Luu-The V. Assessment of steroidogenic pathways that do not require testosterone as intermediate. Horm Mol Biol Clin Invest. 2011; 5:161–165.

43. Chang KH, et al. Dihydrotestosterone synthesis bypasses testosterone to drive castration-resistant prostate cancer. Proc Natl Acad Sci U S A. 2011; 108:13728–13733. [PubMed: 21795608]

44. Knudsen KE, Penning TM. Partners in crime: deregulation of AR activity and androgen synthesis in prostate cancer. Trends Endocrinol Metab. 2010; 21:315–324. [PubMed: 20138542]

45. Efstathiou, E.; T, S.; Aparicio, A.; Hoang, A.; Wen, S.; Troncoso, P.; Smith, LA.; Chieffo, N.; Molina, A.; Logothetis, C. Use of “intracrine androgen signaling signature” to predict benefit from abiraterone acetate (AA) in patients with castrate-resistant prostate cancer (CRPC). 2010 ASCO Annual Meeting; Chicago, IL. 2010.

46. Eisenberger MA, et al. Phase I and clinical pharmacology of a type I and II, 5-alpha-reductase inhibitor (LY320236) in prostate cancer: elevation of estradiol as possible mechanism of action. Urology. 2004; 63:114–119. [PubMed: 14751361]

47. Shah SK, et al. Phase II study of Dutasteride for recurrent prostate cancer during androgen deprivation therapy. J Urol. 2009; 181:621–626. [PubMed: 19091347]

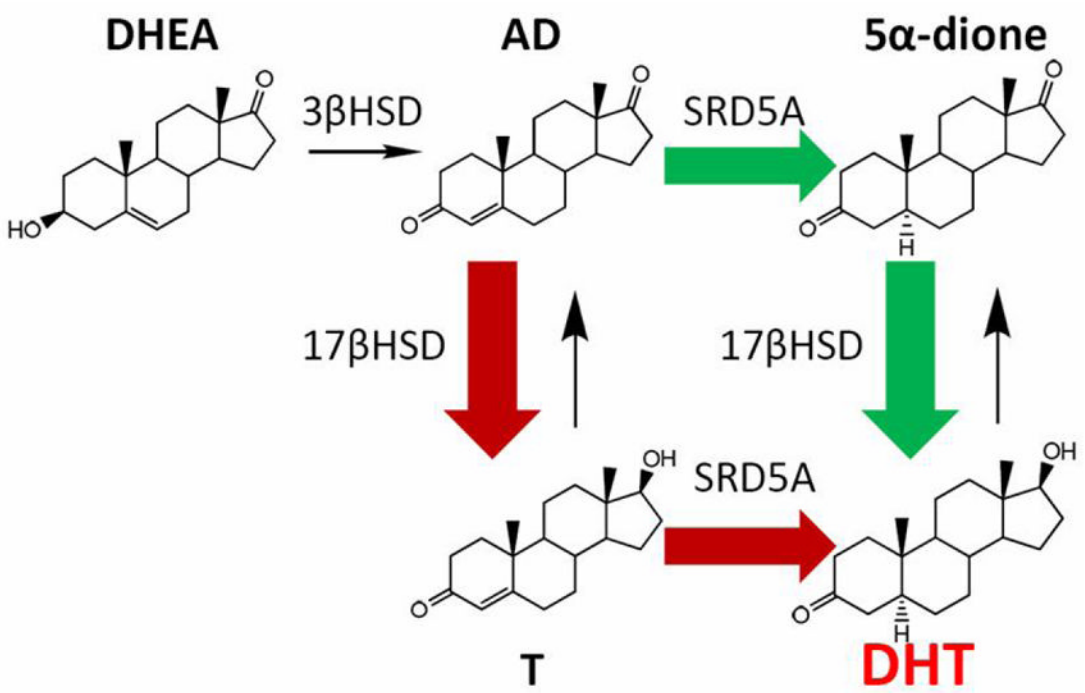

Figure 1.
The pathway overview of DHT synthesis. The synthesis of intratumoral DHT requires enzymatic modification of the 3-, 5-, and 17-positions of the steroid backbone by 3β-hydroxysteroid dehydrogenase (3βHSD), steroid 5α-reductase (SRD5A) and 17β-hydroxysteroid dehydrogenase (17βHSD) isoenzymes. The widely accepted conventional pathway requires conversion of AD to T (red arrows). An alternative possibility circumvents the requirement for T by 5α-reduction of AD to 5α-dione (green arrows).