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TGF β 1 alters androgenic metabolites and hydroxysteroid dehydrogenase enzyme expression in human prostate reactive stromal primary cells: Is steroid metabolism altered by prostate reactive stromal microenvironment?

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Abstract

The inflammatory tissue microenvironment can be an active promoter in preneoplastic cancer lesions. Altered steroid hormone metabolism as induced by the inflammatory microenvironment may contribute to epithelial cancer progression. Dehydroepiandrosterone sulfate (DHEAS) is the most abundant endogenous steroid hormone present in human serum and can be metabolized to DHEA, androgens and/or estrogens in peripheral tissues. We have previously reported that TGF β 1-induced reactive prostate stromal cells increase DHEA metabolism to active androgens and alter prostate cancer cell gene expression. While much of the focus on mechanisms of prostate cancer and steroid metabolism is in the epithelial cancer cells, this study focuses on TGF β 1-induced effects on DHEA metabolic pathways and enzymes in human prostate stromal cells. In DHEA-treated primary prostate stromal cells, TGF β 1 produced time- and dose-dependent increases in metabolism of DHEA to androstenedione and testosterone. Also TGF β 1-treated prostate stromal cells exhibited changes in the gene expression of enzymes involved in steroid metabolism including up-regulation of 3 α hydroxysteroid dehydrogenase (HSD), and down-regulation of 17 β HSD5, and 17 β HSD2. These studies suggest that reactive prostate stroma and the inflammatory microenvironment may contribute to altered steroid metabolism and increased intratumoral androgens.

Keywords

TGF β 1; Reactive prostate stroma; Androstenedione; Testosterone; DHEA; Steroid metabolism

1. Introduction

Interactions between stromal cells and tumor cells are known to play a major role in cancer development. The change of stromal cells from normal status to the inflammatory reactive stage represents a critical step in tumor progression by providing a growth promoting microenvironment that can influence the function of epithelial cells [1]. In the prostate, reactive stromal cells secrete large amounts of growth factors, extracellular matrix components and remodeling enzymes similar to a wound repair response [2,3]. Normal

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stromal cells in the prostate may become reactive in response to inflammatory signals, such as transforming growth factor beta 1 (TGF β 1), a pro-inflammatory cytokine that influences many cellular processes, including proliferation, differentiation and apoptosis and exerts multiple effects on carcinogenesis in the prostate [2,4].

Androgens are crucial steroid hormones for the development and function of the prostate. It is well established that prostate epithelial cells are the primary targets of androgen and the origin of adenocarcinoma that account for over 90% prostate tumors. In the prostate, the most potent form of androgen is dihydrotestosterone (DHT), which partially comes from blood circulation but is mainly produced in the prostate from testosterone (T) by 5 α -reductases [5]. Traditionally, it was thought that the concentration of T in the prostate was determined by the blood levels of T. Accumulating evidence suggests that the prostate is capable of producing T from metabolism of dehydroepiandrosterone (DHEA). In humans the circulating, sulphated form of DHEA, DHEAS, provides a large reservoir of steroid substrate, up to 100–500 times the levels of testosterone and 1000–10,000 times higher than those of estradiol [6,7] for conversion into androgens and/or estrogens in peripheral intracrine tissues. In the prostate, intratissular DHEA contributes up to 1/6 of available DHT [8]. Being a weak androgen, DHEA is predominantly synthesized in the adrenal glands and gonads [9], existing at highest level in the blood in both men and women in their 20's and 30's. Circulating DHEA levels decrease with increasing age, so that by age 70 DHEA levels are decreased by 80% compared to a 25 year old [10]. DHEA is widely available in the US as an over-the-counter dietary supplement and used by older adults for its alleged anabolic and anti-aging effects, while any benefits or long term safety remain uncertain [11]. Long term supplementation is a concern as DHEA could be metabolized to androgens or estrogens in target reproductive tissues.

Metabolism of DHEA is accomplished by a series of steroidogenic enzymes, as illustrated in Fig. 1. DHEA-sulfate (DHEAS) is present in high levels in the prostate, as is the sulfatase that converts DHEAS to DHEA [12]. The production of T in peripheral tissues can be achieved through several pathways: DHEA $\rightarrow$ androstenediol (5-diol) $\rightarrow$ T; or DHEA $\rightarrow$ androstenedione (4-dione) $\rightarrow$ T and as recently described for castration-resistant prostate cancer a pathway that bypasses T synthesis: DHEA $\rightarrow$ (4-dione) $\rightarrow$ 5 α -androstenedione (A-dione) $\rightarrow$ DHT, mediated by steroid-5 α -reductase isoenzyme-1 (SRD5A1) [13]. In the prostate, the first pathway is unlikely active because 17 β -hydroxysteroid dehydrogenase type 1 (17 β -HSD1) is poorly expressed by prostate tissues [14]. In the second pathway, 3 β -HSDs convert DHEA to 4-dione and reductive 17 β -HSDs, such as 17 β -HSD5 (also known as AKR1C3) catalyze the conversion of 4-dione to T. These enzymes are present in the prostate and believed to be responsible for formation of 4-dione and T as well as the presence of aromatase which can further metabolize 4-dione $\rightarrow$ estrone (E1) or T $\rightarrow$ estradiol (E2) [15]. While the regulatory factors affecting expression of T or E synthesizing enzymes in the prostate remain unclear, we present data on a factor common in the inflammatory microenvironment, TGF β 1, which may regulate DHEA metabolism specifically in the stromal cell compartment. Our hypothesis is that the inflammatory microenvironment via paracrine effects may alter androgen/estrogen steroid balance in the prostate and be an important preneoplastic mechanism contributing to prostate cancer promotion.

TGF β 1 is known to influence steroid biosynthesis in adrenocortical cells by decreasing 3 β -HSD expression [16–18]. In breast cancer cells, it enhances the action of reductive 17 β -HSD activity [19,20]. In the prostate, TGF β 1 acts as a potent pro-inflammatory factor that induces the formation of reactive stroma cells [4]. We have previously shown that prostate stromal cells are capable of metabolizing DHEA to T in response to TGF β 1 [21]. This study expounds the role of TGF β 1 as a significant regulator of T biosynthesis in the prostate

primary stromal cells including the time and dose responsiveness of TGF β 1 induction of DHEA metabolites and expression of responsible HSD's. This work emphasizes the role of stromal metabolism of DHEA to androgenic ligands in prostate tissues which is subject to tissue microenvironmental factors of inflammation as represented by TGF β 1.

2. Materials and methods

2.1. Cell culture

Two types of primary human prostate stromal cells were utilized in the present study. These included primary stromal cells derived from radical prostatectomy tissues, named 6S cells [22,23] and primary prostate stromal cells, PrSC, obtained from Lonza (Walkersville, MD). Human prostate cancer LAPC-4 cells were obtained as previously described [21,24]. All cell types were maintained in DMEM:F12 (1:1) medium (Invitrogen, Gaithersburg, MD) containing penicillin (100 units/mL), streptomycin (100 μ g/mL), L-glutamine (292 μ g/mL) (Invitrogen), and 5% fetal bovine serum (FBS, HyClone Laboratories, Inc., Logan, UT) at 37 °C in 5% CO₂.

Stromal cells were kept as frozen stocks in liquid nitrogen and used within seven passages after thawing. The experiments were performed in treatment medium consisting of 50% Medium 199 (phenol red-free) and 50% F12 phenol red-reduced media (Invitrogen, Carlsbad, CA) supplemented with penicillin (100 units/ml), streptomycin (100 mg/ml), glutamine (100 mg/ml; Invitrogen) and 1% charcoal-dextran-treated FBS (CDS – Hyclone Labs).

2.2. ELISA analysis of testosterone and androstenedione

Stromal cells (6S or PrSC) were plated in triplicate at 6.0×10^5 cells/plate in 60-mm plates or 2.0×10^5 cells/well in 6-well plates, or 1×10^5 cells per well in triplicate in 12 well plates and cultured with Treatment Media (as previously defined [24]) for 24 h before hormone or TGF β 1 treatment. LAPC-4 cells were plated as above but at densities 4 $\times$ that of the stromal cells. DHEA (100 nM) or 4-dione (10 nM) was added to treatment medium for the indicated time points with or without TGF β 1. The conditioned culture media was collected for ELISA analysis of DHEA metabolites and the cells were subject to RNA isolation. DHEA metabolites were detected by using ELISA kits for free testosterone (cat # 11-FTSHU-E01-ALPCO, Salem, NH) and androstenedione (cat # DSL-10-3800-Beckman Coulter DSLabs, Webster, TX) according to the manufacturer's instructions. Each original triplicate experimental sample was analyzed in duplicate. Values were corrected for relative cell numbers as estimated using the CellTiter 96® Assay (MTT assay) (Promega, Madison, WI), as previously described [22].

2.3. RNA isolation and real-time RT-PCR

Total RNA from cultured cells 6S and LAPC4 was isolated using TRIzol reagent (Invitrogen) according to manufacturer's instruction with the following modification. Before RNA precipitation the samples were extracted with equal volume of phenol/chloroform and then chloroform. The extracted samples were precipitated with 0.8 volume of isopropanol followed by two washes with 70% ethanol. RNAs were dissolved in DEPC-treated H₂O at concentration of 1 μ g/ μ l after quantification by a spectrophotometer at 260 nm. The reverse transcription was performed with Superscript III RNase HRT (Invitrogen) as described by [22]. Each reaction contained 1.0 μ g of total RNA, 150 ng of random hexamer (Amersham Biosciences), 50 units of Superscript III and 10 units of RNasin (Promega). PCR was performed using TaqMan probes (see list below) and Gene Expression Master Mix (Applied Biosystems, Foster City, CA)

- TaqMan® Gene Expression Assay (Hs00157993_m1): HSD17B2.
- TaqMan® Gene Expression Assay (Hs00426435_m1): HSD3B1.
- TaqMan® Gene Expression Assay (Hs00605123_m1): HSD3B2.
- TaqMan® Gene Expression Assay (Hs00366267_m1): HSD17B5.

Gene expression was quantitated by a 7900 HT Fast Real-Time PCR System (Applied Biosystems) according to the manufacturer's manual. Relative mRNA expression levels were calculated using the $\Delta\Delta CT$ (Delta Delta CT) method ($N_{\text{target}} = 2^{(Ct_{\text{sample}} - Ct_{\text{calibrator}})}$ [25]) to provide relative gene expression where the calibrator was the GAPDH gene.

2.4. Statistical analysis

The results are presented as the mean $\pm$ SD obtained from three independent experiments, with at least three sample replicates in each. All data were analyzed by Student's *t*-test or one way analysis of variance (ANOVA) where appropriate. An adjusted *P*-value of 0.05 was considered significant.

3. Results

3.1. DHEA is metabolized by prostate stromal cells to 4-dione and testosterone

Prostate stromal cells that were cultured in the presence of DHEA had increased levels of, both 4-dione and T in conditioned media as detected by ELISA (Fig. 2A + B – hatched control bars). Both androgens were detected at 6 h, and their concentrations progressively increased over time. At each time point, the levels of 4-dione was increased as compared to T. At 48 h, for example, the ratio of 4-dione over T was 1:2.5 and at 72 h was 1:2.9 ($p < 0.05$).

3.2. TGF β 1-treatment increases metabolism of DHEA to 4-dione and T production in cultured prostate stromal cells: time and dose responses

To test the timing of the effect of TGF β 1 on DHEA metabolism in prostate stromal cells, 6S cells were cultured and treated with 100 nM DHEA and 100 pM TGF β 1 for 6–72 h, and the conditioned media were collected for the detection of 4-dione (Fig. 2A) and T (Fig. 2B) by ELISAs. TGF β 1 increased the production of 4-dione by 1.5 fold ($p < 0.05$) at 12 h, and further elevated the production of 4-dione to 2.0 ($p < 0.001$), 3.4 ($p < 0.001$) and 3.4-fold ($p < 0.001$) at 24, 48 and 72 h, respectively. Also the production of T was induced by TGF β 1 in a time-dependent manner. At 48 and 72 h, the induction was 3.3 ($p < 0.001$) and 4.1-fold ($p < 0.001$), respectively. Parallel plates were prepared for determination of cell growth or inhibition by DHEA or TGF β 1 treatments. Since stromal cells were regularly plated at near confluence, neither treatment at any dose used altered final cell numbers and were not significant variables for ELISA (data not shown).

The dose effect of TGF β 1 on DHEA metabolism was also investigated by treating 6S cells, in the presence of 100 nM DHEA, with TGF β 1 at a range of 10–200 pM for 48 h. TGF β 1 (25 pM) stimulated the conversion of DHEA to 4-dione by 1.9-fold ($p < 0.001$) (Fig. 3A). The extent of induction was progressively increased with higher doses of TGF β 1, reaching 3.3-fold ($p < 0.01$) with 200 pM TGF β 1.

Similarly, DHEA $\rightarrow$ T production was stimulated 1.9-fold ($p < 0.001$) when cells were treated with 25 pM of TGF β 1 for 48 h (Fig. 3B), and by 3.3-fold ($p < 0.001$) with 200 pM TGF β 1. Finally, TGF β 1 effects on 4-dione $\rightarrow$ T were measured by treating 6S cells with TGF β 1 plus 4-dione (10 nM) and conditioned media was collected for ELISA determination of T. TGF β 1 moderately increased the conversion of 4-dione to T in a dose-dependent

manner (Fig. 3C). The induction folds were 1.3, ($p < 0.05$) 1.5 ($p < 0.01$) and 1.7 ($p < 0.01$) when 50, 100 and 200 pM of TGF β 1 were added in the presence of 10 nM 4-dione for 48 h.

3.3. Effects of TGF β 1 on DHEA metabolism in other prostate cells

The action of TGF β 1 on DHEA metabolism in prostate stromal cells (PrSC) from normal prostate was also examined by ELISA. When PrSC were treated with 100 nM DHEA plus 100 pM of TGF β 1, production of both 4-dione and T, were increased by 2-fold ($p < 0.01$) after 48 h compared to treatment with DHEA-alone controls (Fig. 4A and B). In LAPC4 prostate cancer cells, under the same culture and treatment conditions, TGF β 1 did not induce the conversion of DHEA to 4-dione or T (Fig. 4C and D).

3.4. TGF β 1 induction of steroid metabolizing enzymes HSDs in prostate stromal cells

3.4.1. Regulation of stromal 3 β -HSD1 gene expression by TGF β 1—The observation that TGF β 1 increased conversion of DHEA to 4-dione led us to examine whether gene expression of the steroid metabolizing enzyme, 3 β -HSD1, was also regulated by TGF β 1. Real-time RT-PCR analysis was performed with RNA isolated from 6S cells after treatment with TGF β 1 for 48 h. As shown in Fig. 5A, 3 β -HSD1 expression was stimulated by TGF β 1 in a dose-dependent manner, with a range of 4- to 23-fold induction ($p < 0.001$) from 10 to 200 nM TGF β 1. 3 β -HSD2 mRNA was not detectable in RNA samples from 6S cells with or without TGF β 1 treatment (data not shown).

3.4.2. Regulation of stromal 17 β -HSD5 and 17 β -HSD2 gene expression by TGF β 1—Conversion of 4-dione to T involves the actions of at least two enzymes, 17 β -HSD5 that converts 4-dione to T and 17 β -HSD2, which catalyzes the reverse reaction of T to 4-dione. To better understand the role of these two enzymes in TGF β 1-regulated T biosynthesis, real-time RT-PCR analysis was performed with RNA isolated from 6S cells after treatment with DHEA plus TGF β 1 for 48 h. As shown in Fig. 5B and C, the expression of both 17 β -HSD5 and 17 β -HSD2 were down-regulated by TGF β 1 in a dose-dependent manner. TGF β 1 at 100 pM, decreased 17 β -HSD5 expression by 38% ($p < 0.05$), and 17 β -HSD2 expression by 69% ($p < 0.01$), as compared to vehicle. With 200 pM TGF β 1 the 17 β -HSD5 level was reduced by 52.5% ($p < 0.001$), whereas 17 β -HSD2 expression was inhibited 96.5% ($p < 0.001$).

4. Discussion

Paracrine effects of prostate stromal cells on neighboring epithelial cells include localized secretion of growth factors and extracellular matrix products [22,26]. Additionally, this study suggests that prostate stromal cells are an important source of intraprostatic androgens via intracrine metabolism of endogenous DHEA. This study expands our previous work in cocultures of prostate stromal and epithelial cells (LAPC4) where DHEA increased LAPC4 PSA production only in presence of the stromal cells due to stromal capacity to metabolize the DHEA to androgens [27].

TGF β 1 acts as a pro-inflammatory cytokine and induces a reactive phenotype in the stromal cells. In the presence of DHEA, the TGF β 1-induced reactive stroma further amplified the androgenic response in cocultured prostate cancer epithelial cells [24]. We herein validate that in the stromal cells, TGF β 1 increases the conversion of DHEA to both 4-dione and T in prostate stromal cell lots, 6S and PrSC, with no significant change in steroid metabolism in the LAPC4 cancer cells. TGF β 1 also increased the intermediate conversion of 4-dione to T (Fig 3C) and altered the gene expression of steroid metabolizing enzymes, increasing 3 β -HSD1, and decreasing 17 β -HSD5 (a.k.a. ACR1C3) and 17 β -HSD2. This indicates that steroid metabolism can be altered by the prostate reactive stromal microenvironment.

Chronic infection or inflammation is associated with ~20% of human cancers [28]. In the prostate, proliferative inflammatory atrophy (PIA) lesions illustrate the association between inflammation and unusually high proliferation [29]. This preclinical study contributes the additional potential of increased stromal metabolism of endogenous DHEA within the reactive microenvironment, either to androgens or estrogens, along with increased induction of paracrine factors that induce proliferation or inhibit apoptosis. It would be valuable to survey prostate tissues to validate, the coexpression of TGF β 1, increased intraprostatic androgens, in the context of inflammation.

Our knowledge of steroid metabolism effects in prostate cancer progression has been expanded by the discovery of steroidogenic enzymes in peripheral tissues. It is estimated that approximately 40% of the androgens in men are synthesized in peripheral target tissues from adrenal-derived precursors, such as DHEA, that exist at high levels in humans [6]. One pathway of DHT synthesis in the prostate is DHEA $\rightarrow$ 4-dione $\rightarrow$ T through SRD5A2 DHT, in which T is an intermediate product that is further catalyzed to DHT by 5 α -reductases (Fig. 1; also [30]). An alternative pathway that does not require T formation (DHEA $\rightarrow$ 4-dione $\rightarrow$ 5 α -dione $\rightarrow$ DHT) has been proposed [31] and is important in CPRC tissues in presence of SRD5A1 [13]. Steroid metabolism pathways in prostate have been determined in studies of cancer epithelial cells. We add to the literature an important source of prostatic androgens is also produced in prostate stromal cells via metabolism of DHEA and is amplified in the presence of TGF β 1. We hypothesize that tissue microenvironmental factors may alter steroid metabolizing enzymes and androgen biosynthesis in the prostate. 3 β -HSDs are responsible for conversion of DHEA to 4-dione, the first step of DHEA metabolism. Of the two isoforms, 3 β -HSD1 and 3 β -HSD2, the expression of 3 β -HSD1 has been found in peripheral prostate epithelial and stromal cells, although the expression level in epithelial cells was higher [32]. 17 β -HSD5 has been localized in prostate stromal cells by immunohistochemistry [33–35]. It appears that stromal cells contribute at least a portion of the local T production in the prostate.

In this study, we demonstrate that cultured primary prostate stromal cells (6S cells and PrSC) are able to convert DHEA to both 4-dione and T, demonstrating that stromal cells possess the appropriate metabolizing enzymes for T production in the prostate. Accordingly, our analysis by RT-PCR revealed that both 3 β -HSD1 and 17 β -HSD5 (and 17 β -HSD2) were present in 6S cells and PrSCs (data from PrSCs not shown). LAPC4 cells also contain steroid metabolizing enzymes [13,36,37] and as indicated in Fig. 3C and D where >4 ng/mL T was found in DHEA-treated LAPC4 cells. TGF β 1 did not significantly increase T and 4-dione production in LAPC4 cells. In prostate tissues steroid metabolism occurs in both stromal and epithelial components [38]. Our results were limited to analysis of steroid metabolites by ELISA. The use of HPLC or LC-MS would better determine the degree of resolution of steroid metabolites and would increase ability to detect DHT levels, which, in our lab, had too much cross-reactivity from other steroid signals.

TGF β 1 exerts a range of cellular and phenotypic effects in the induction of reactive stroma in prostate [4]. We suggest that an additional critical component of reactive stroma is altered steroid metabolism. The effects of TGF β 1 on steroid hormone metabolism may provide a link between reactive stroma, the inflammatory microenvironment, and androgen production. TGF β 1 effects on steroid metabolizing enzymes varies in different tissues. For example TGF β 1 down-regulated 3 β -HSD activity and adrenal steroid production in adrenocortical cells [16–18]. It also inhibited progesterone synthesis induced by luteinizing hormone (LH) in cultured porcine ovarian thecal cells [39]. In contrast, TGF β 1 was found to stimulate 3 β -HSD1 activity in porcine Leydig cells [40]. In the current study, the stimulatory effect of TGF β 1+ DHEA on 3 β -HSD1 expression was validated in prostate stromal (6S) cells using real-time RT-PCR where increasing doses of TGF β 1 along with 100 nM DHEA

significantly increased 3 β -HSD1 mRNA expression. The level of 3 β -HSD2 mRNA remained undetectable after treatment with TGF β 1 (data not shown).

The oxidative enzyme 17 β -HSD2 catalyzes the conversion of 17 β -hydroxysteroids to 17-ketosteroids and is reported to be present in prostate epithelial cells [14]. It was proposed that the expression of 17 β -HSD2 may protect hormone target tissues from excessive sex steroid influence by catalyzing the conversion of androgens and estrogens to less active forms [5]. Further, Li and colleagues demonstrated that 17 β -HSD2 is present in stromal cells of placental villi, where it restricts the access of the active estrogen into fetal circulation by converting estradiol to estrone [41]. In the prostate stromal cells, TGF β 1 down-regulated both 17 β -HSD5 and 17 β -HSD2, which catalyze opposite reactions. The activity of 17 β -HSD2 in stromal cells may convert T back to 4-dione. At the dose of 100 pM used in these studies, TGF β 1 decreased 17 β -HSD2 expression to a greater degree than 17 β -HSD5, resulting in overall increased conversion of 4-dione to T. A similar effect was also observed in endometrial cancer cells where TGF β 1 decreased 17 β -HSD2 expression [42]. We do not understand all the mechanisms behind TGF β 1 regulation of 3 β -HSD1, 17 β -HSD2 and 17 β -HSD5, but appreciate this example of how tissue microenvironmental alterations may contribute to changes in steroid hormone levels in tissues.

Important and still unexplored are pathways of DHEA metabolism to estrogen metabolites in the prostate. Aromatase was present in the 6S prostate stromal cells, (see also [15]). In preliminary studies, we found TGF β 1 decreased metabolism of DHEA $\rightarrow$ E1 and decreased stromal aromatase expression (by RT-PCR) with no significant change in DHEA $\rightarrow$ E2 (see supplemental data).

See Supplementary Fig. 1 as supplementary file. Supplementary material related to this article found, in the online version, at <http://dx.doi.org/10.1016/j.jsbmb.2013.05.016>.

This data supports our previously described model of endocrine-immuno-paracrine interactions [43,44] in the prostate, by defining the influence of TGF β 1 on the alteration of the stromal cell metabolic machinery. We have recently developed a cost effective bioassay in which phytoestrogens alone or in mixtures reversed TGF β 1-induced androgen metabolism and paracrine epithelial PSA production [24,44]. Natural compounds or prostate cancer drugs that target steroid metabolism, including abiraterone which blocks CYP17A1 – an enzyme upstream of DHEA [45] should include additional targets that can reverse TGF β 1 effects on steroid enzymes, normalize reactive stroma and prevent prostate cancer progression.

5. Conclusion

This work details TGF β 1 induction of metabolism of DHEA to androstenedione and testosterone in human primary prostate stromal cells, outlining dose and time dependent increases in the steroid metabolites as well as changes in the gene expression of steroid metabolism enzymes 3 β -HSD1, 17 β -HSD2 and 17 β -HSD5. Prostate cancer cells (LAPC-4) did not show similar induction of hormone metabolism with TGF β 1 treatment. Human prostate reactive stromal cells are an important contributor to androgen metabolism in the prostate inflammatory microenvironment as induced by local cytokines such as TGF β 1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

6S	cancer-derived primary stromal cells
A-dione	5 -androstanedione
5-diol	androstenediol
4-dione	androstenedione
ANOVA	analysis of variance
AR	androgen receptor
DHEA	dehydroepiandrosterone
DHEAS	DHEA sulfate
DHT	dihydrotestosterone
DMEM	Dulbecco's modified Eagles media
ELISA	enzyme-linked immunosorbent assay
E1	estrone
E2	estradiol
FBS	fetal bovine serum
HSD	hydroxysteroid dehydrogenase
17HSD2 or 17HSD5	17 -hydroxysteroid dehydrogenase type 2 or type 5
PrSC	normal primary prostate stromal cells
S.E.M.	standard error of the mean
T	testosterone
TGF 1	transforming growth factor -1

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DHEA Metabolic Pathways in the Prostate

Fig. 1.

DHEA metabolic pathways in the prostate and in cultured 6S cells. Metabolism of DHEA in the prostate toward androgens or estrogens depends on activity of steroid metabolism enzymes including: hydroxysteroid dehydrogenase (HSD), alpha-keto reductase (AKR), steroid reductase dehydrogenase 5 alpha 1,2 (SRD5a1,2), sulfatase (STS), sulfonotransferase (SULT2A1) and aromatase. DHEA androgenic or estrogenic metabolites include: dehydroepiandrosterone (DHEA), DHEA sulphate (DHEA-S), androstenediol (5-diol), 4-androstenedione (4-dione), testosterone (T), estrone (E1), estradiol (E2), 5 - androstenedione (A-dione), dihydrotestosterone (DHT), androsterone (ADT), 3 - andostanediol (3 -diol), 3 -androstenediol (3 -diol).

Adapted from Luu-The, 2008 and Penning, 2009.

Fig. 2.

DHEA metabolism in prostate stromal (6S) cells: TGF β 1 induced increase in 4-dione and T production in DHEA-treated prostate stromal 6S cells over time. Cells were plated onto 60-mm plates at a density of 6.0×10^5 cells/plate with normal medium. After 24 h, normal medium was replaced by 3 mL treatment medium (see Section 2) and the cells were cultured for further 24 h before stimulation. The medium was replaced with 2.5 mL of new treatment medium containing 100 nM DHEA plus or minus 100 pM TGF β 1, and the cells were cultured for 6, 12, 24, 48 and 72 h before the media were collected. The amount of 4-dione (A) and T (B) in the media was analyzed by ELISA. Control values of stromal metabolism are represented by hatched bars. Solid bars represent induction of 4-dione or T by TGF β 1. The results represent mean $\pm$ SD obtained from three independent experiments, with triplicate samples in each. * $P < 0.05$, *** $P < 0.001$; treated compared to control at each time point.

Fig. 3.

Effect of increasing doses of TGF β 1 on 4-dione and T production in prostate stromal 6S cells. 6S cells were plated onto 60-mm plates at a density of 6.0×10^5 cells/plate with normal medium. After 24 h the medium was replaced with 2.5 mL new treatment medium containing 100 nM DHEA and 10–200 pM TGF β 1, and the cells were cultured for 48 h before the media was collected for ELISA of 4-dione (A) and T (B). To distinguish action of TGF β 1 on the conversion of 4-dione to T in 6S cells (3C), cells were treated as above then final treatment consisted of 10 nM 4-dione and 100 pM TGF β 1, for 48 h. Conditioned media were collected and T concentration was measured by ELISA. All results shown in A–C represent mean $\pm$ SD obtained from three independent experiments, with triplicate in each. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 4.

Effects of TGF β 1 on DHEA metabolism in PrSC and LAPC4 cells. Normal primary stromal cells (PrSC) cells were plated onto 60-mm plates at a density of 6.0×10^5 cells/plate (or 2×10^6 for LAPC4 cells) with normal medium and allowed to attach for 24 h (PrSC; A and B) or 48 h (LAPC4 cells; C and D). Then, normal medium was replaced by 3 mL treatment medium, and the cells were cultured for further 24 h. Cells were then treated with 2.5 mL of new treatment medium consisting of 100 nM DHEA or DHEA plus 100 pM TGF β 1 for 48 h. The media were collected and the concentrations of 4-dione (A and C) and T (B and D) were accessed by ELISA. All results shown represent mean $\pm$ SD obtained from three independent experiments, with triplicate in each *** $P < 0.001$.

Fig. 5.

Regulation of HSD gene expression by TGF 1. 6S cells were plated at a density of 6.0×10^5 cells/plate onto 60-mm plates with normal medium. After 24 h, medium were replaced by 3 ml treatment medium and the cells were cultured for further 24 h. Then the cells were stimulated by replacing the medium with 2.5 ml of new treatment medium containing 100 nM DHEA and 10–200 pM TGF 1. After treatment for 48 h, total RNAs were isolated from the cells using TRIzol reagent. Performance of reverse transcription is described in Section 2. For expression of 3 HSD1(A), 0.5 μ l of cDNA was used for each real-time PCR reaction. For expression of 17HSD5 (B) and 17HSD2 (C) 1.0 μ l of cDNA was used for each real-time PCR reaction. The fold changes in the amount of mRNAs are represented as mean $\pm$ SD obtained from three independent experiments, with triplicate in each. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.