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Genes and proteins of the alternative steroid backdoor pathway for dihydrotestosterone synthesis are expressed in the human ovary and seem enhanced in the polycystic ovary syndrome

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ABSTRACT

Recently, dihydrotestosterone biosynthesis through the backdoor pathway has been implicated for the human testis in addition to the classic pathway for testosterone (T) synthesis. In the human ovary, androgen precursors are crucial for estrogen synthesis and hyperandrogenism in pathologies such as the polycystic ovary syndrome is partially due to ovarian overproduction. However, a role for the backdoor pathway is only established for the testis and the adrenal, but not for the human ovary. To investigate whether the backdoor pathway exists in normal and PCOS ovaries, we performed specific gene and protein expression studies on ovarian tissues.

We found aldo-keto reductases (AKR1C1-1C4), 5α-reductases (SRD5A1/2) and retinol dehydrogenase (RoDH) expressed in the human ovary, indicating that the ovary might produce dihydrotestosterone via the backdoor pathway. Immunohistochemical studies showed specific localization of these proteins to the theca cells. PCOS ovaries show enhanced expression, what may account for the hyperandrogenism.

1. Introduction

Androgens are produced from cholesterol in the human gonads and in the adrenal cortex. They are mainly known for their masculine effects, which are principally exerted through transcriptional regulation via androgen receptors at the cellular level. Androgen action is needed very early in fetal life for male sexual development, during adolescent years for pubertal development and in adulthood for male fertility and reproduction. Similar to males, the female development, fertility and reproduction are equally dependent on androgens. Not only are androgens essential precursors for all estrogen biosynthesis, but also crucial for the normal development of ovarian follicles as shown by knockout of the androgen receptor in mice (Hu et al., 2004; Shiina et al., 2006). In addition, hyperandrogenism in women is associated with disease states such as the polycystic ovary syndrome (PCOS). PCOS is defined by androgen excess, menstrual abnormalities and polycystic ovaries. Although as many as 10% of women are affected by the PCOS and large efforts in terms of clinical and basic studies are ongoing, its pathophysiology is still poorly understood and therapeutic options are scarce. As androgen biosynthesis plays a pivotal role in ovarian physiology, a better understanding of androgen biosynthesis and its regulation may help in understanding this reproductive disorder, which is the most frequent cause of anovulation and infertility.

Though the biochemistry and the genetics of testosterone and dihydrotestosterone (DHT) synthesis from cholesterol have been described decades ago, only recent work has revealed a second pathway for DHT biosynthesis, which is now known in the

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literature as the alternative, backdoor pathway, to distinguish it from the classic pathway (Auchus, 2004 and Fig. 1). In contrast to the classical pathway, DHT synthesis through the backdoor pathway does not proceed through testosterone, but diverges off at the level of 17-hydroxyprogesterone (17OHP) and proceeds through 17OH-dihydroprogesterone (17-DHP), 17OH-allopregnanolone (17-Allo), androsterone and androstanediol (3αDiol) to form the most potent androgen DHT. Enzymes involved in the backdoor pathway are 5α-reductase type 1 (SRD5A1), aldo-keto reductase family 1 members C2/C4 (AKR1C2/C4), cytochrome P450c17 (CYP17A1), aldo-keto reductase family 1 C3 (AKR1C3)/17β-hydroxysteroid dehydrogenase type 5 (HSD17B5) and retinol dehydrogenase (RoDH)/17β-hydroxysteroid dehydrogenase type 6 (HSD17B6) (Auchus, 2004; Miller and Auchus, 2011) (Table 1). Regarding CYP17A1, its enzyme activity in the backdoor pathway is in contrast to the classic pathway not dependent from cytochrome b5. In addition to the classic and alternative backdoor pathway, Fassnacht et al. showed a third, androstanedione-mediated route to DHT (Fassnacht et al., 2003). This route of DHT formation from androstenedione via 5α-androstanedione is dominant in castration-resistant prostate cancer (Chang et al., 2011).

The backdoor pathway was first discovered in the testes of the tammar wallaby pouch young, where androstanediol is formed by a pathway involving 17OH-Allo as a key intermediate (Wilson et al., 2003). Accordingly, analysis of urine steroid profiles of patients presenting with features of hyperandrogenism due to cytochrome P450 oxidoreductase (POR) deficiency revealed steroid metabolites implicating the backdoor pathway in humans (Homma et al., 2006). Furthermore, human mutations in the aldo-keto reductase genes AKR1C2 and AKR1C4, both specific for the backdoor pathway, were found in 46,XY individuals with disordered sexual development, illustrating that both the classic and the backdoor pathways are needed for normal male fetal androgen biosynthesis in the testis (Fluck et al., 2011). In addition, activity of the backdoor path was observed in patients with 21-hydroxylase deficiency, establishing a role of the backdoor pathway for the human adrenal cortex (Kamrath et al., 2012).

Overall, the steroid backdoor pathway for androgen biosynthesis plays a role in the normal human adrenal cortex and the testis, and it seems to play an important role in steroid disorders. However, its role in the human ovary remains elusive. Currently, circulating DHT in females is mostly assigned to peripheral conversion of precursor steroids (Miller and Auchus, 2011). The contribution of ovarian DHT production, either by stepwise conversion from cholesterol or by conversion of steroid metabolites circulating back from the periphery, is unknown. Therefore, this study aimed to establish the role of the backdoor androgen biosynthesis pathway for the human ovary itself. To illustrate the backdoor path in the female gonad, we performed gene and protein expression studies of the specific enzymes of the backdoor pathway in human ovarian tissues. Tissues of human adrenals and testes served as controls. In addition, we compared ovarian tissues of healthy females with tissues of PCOS females. Using this approach we show that the human ovary expresses all genes necessary for DHT production via the backdoor pathway. Interestingly, alterations in the expression of alternative backdoor pathway genes were found in PCOS when compared to controls suggesting that androgen excess in PCOS may (at least in part or in some PCOS patients) originate from the backdoor pathway.

2. Materials and methods

2.1. Tissue samples

Pathway analysis was performed on fresh frozen and formalin fixed and paraffin embedded (FFPE) tissue samples of human adrenals, testes and ovaries. Samples were obtained from the ethically approved biobank of the Institute of Pathology Bern and the Faculty of Medicine Bern (Bern, Switzerland). Samples were selected by histological evaluation, as well as evaluation of patients' medical data. PCOS samples were chosen according the Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Group and included tissue samples of women suffering from two of the following three criteria: one, clinical and/or biochemical hyperandrogenism, two,

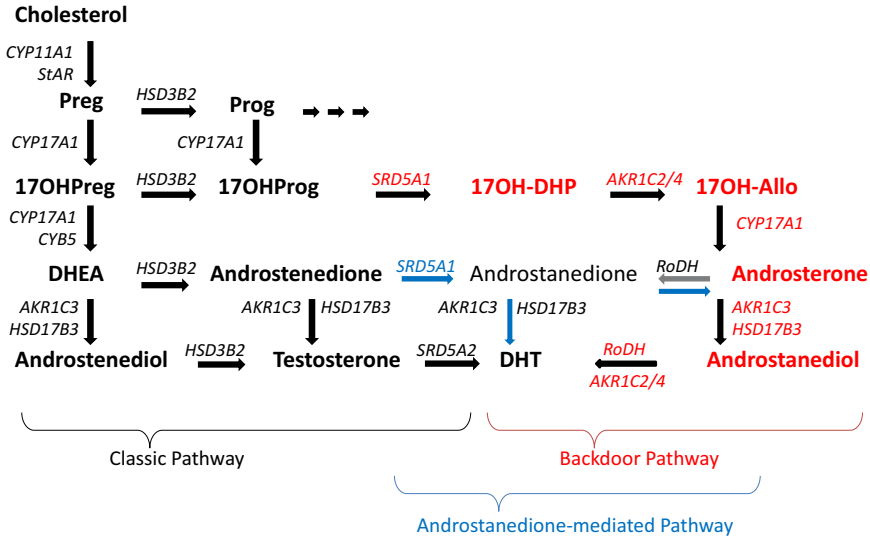

Fig. 1. The classic and the alternative backdoor pathway for androgen biosynthesis. Dihydrotestosterone (DHT) is produced from cholesterol by two pathways, which diverge at the level of 17-hydroxyprogesterone (17OHProg). The diagram illustrates the two distinct routes depicting the intermediates and enzymes involved in both pathways. In addition, the diagram shows a third pathway from DHEA to DHT, the so-called non-conventional androstanedione-mediated pathway (17). See also Table 1. Preg, pregnenolone; Prog, progesterone; 17OHPreg, 17α-hydroxypregnenolone; 17OH-DHP, 17-hydroxy-dihydroprogesterone; 17OH-Allo, 17-hydroxy-allopregnanolone; DHT, dihydrotestosterone; CYP11A1, cholesterol side-chain cleavage enzyme, P450scc; StAR, steroidogenic acute regulatory protein; CYP17A1, 17α-hydroxylase/17,20-lyase; HSD3B2, 3β-hydroxysteroid dehydrogenase, type 2; HSD17B3/5, 17β-hyroxysteroid dehydrogenase, type 3/5; SRD5A1/2, 5α-reductase, type 1/2; AKR1C2/3/4, aldo-keto reductase family 1, member C2/C3/C4; RoDH, retinol dehydrogenase/17β-hydroxysteroid dehydrogenase, type 6; CYB5, cytochrome b5.