In situ androgen and estrogen biosynthesis in endometrial cancer: focus on androgen actions and intratumoral production

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Abstract

In situ estrogen biosynthesis is considered to play pivotal roles in the development and progression of human endometrial carcinoma. However, the biological roles of androgen have remained virtually unknown. Various epidemiological studies have revealed that elevated serum androgen levels are generally associated with an increased risk of developing endometrial carcinoma; however, studies directly examining androgens in carcinoma tissues are relatively rare and reviews summarizing this information are scarce. Therefore, we summarized recent studies on androgens in endometrial carcinoma, especially focusing androgen actions and *in situ* androgen biosynthesis. Among the enzymes required for local biosynthesis of androgen, 17β-hydroxysteroid dehydrogenase type 5 (conversion from androstenedione to testosterone) and 5α-reductase (reduction of testosterone to dihydrotestosterone (DHT)) are the principal enzymes involved in the formation of biologically most potent androgen, DHT. Both enzymes and androgen receptor were expressed in endometrial carcinoma tissues, and *in situ* production of DHT has been reported to exist in endometrial carcinoma tissues. However, testosterone is not only a precursor of DHT production, but also a precursor of estradiol synthesis, as a substrate of the aromatase enzyme. Therefore, aromatase could be another key enzyme serving as a negative regulator for *in situ* production of DHT by reducing amounts of the precursor. In an *in vitro* study, DHT was reported to exert antiproliferative effects on endometrial carcinoma cells. Intracrine mechanisms of androgens, the downstream signals of AR, which are directly related to anticancer progression, and the clinical significance of DHT-AR pathway in the patients with endometrial carcinoma have, however, not been fully elucidated.

Key Words

- androgen
- androgen receptor
- endometrial carcinoma
- 5α-dihydrotestosterone
- 5α-reductase
- aromatase

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Introduction

Endometrial carcinoma is one of the most common female pelvic malignancies in the developed countries, and its incidence has increased. In the United States, 60,050 new cases and 10,470 deaths due to endometrial carcinoma were reported in 2016 (Siegel *et al*. 2016). It is well known that sex-steroid hormones play pivotal roles

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in the development of hormone-dependent carcinoma, including prostate, breast and endometrium, and in the case of the latter, estrogen actions are well known to play important role in the development and progression of endometrial carcinoma. Results of many previously reported clinical studies demonstrated that excessive and/or prolonged exposure to unopposed estrogens increased the risk of endometrial carcinoma especially that of the endometrioid type, also known as type I endometrial carcinoma (Bokhman 1983). However, in contrast to studies regarding estrogens, the possible impact of androgen actions on endometrial carcinoma has largely remained unknown. Therefore, we summarize the recent studies regarding the effects and/or actions of androgens in endometrial carcinoma in this review.

Serum androgen and increased risk of developing endometrial cancer

The great majority of endometrial cancers arise during the postmenopausal period. Circulating estrogen levels decline steeply after menopause but testosterone levels remained unchanged during the menopausal years (Burger *et al.* 2000). Over the age of 65 years, total testosterone levels decline with age until 80, alongside free testosterone levels did not vary by age (Cappola *et al.* 2007). All androgens in postmenopausal women are suggested to be made locally in peripheral target tissues according to the mechanism of local intracrine formation of steroids. The contribution of ovarian production of circulating androgenic precursors and thus potentially supporting this local intracrine metabolism of steroids is underlined by studies showing serum dehydroepiandrosterone (DHEA); the inactive precursor hormone of androgens is higher in intact compared with oophorectomized women (Labrie *et al.* 2011, Labrie 2015), and several studies reported that the serum levels of androgens between ovarian and peripheral veins were significantly different between early and late menopausal women, suggesting that the production of androgens by the ovaries could persist even 10 years after the onset of menopause, but may subsequently decline (Fogle *et al.* 2007, Maruoka *et al.* 2014). Therefore, the degree and duration to which postmenopausal ovaries remain a viable androgen-producing organ is still controversial and not fully understood.

Several investigators reported that elevated serum levels of androgen, including DHEA, DHEA sulfate (DHEAS), androstenedione (A4), testosterone and 5α-dihydrotestosterone (DHT), were significantly associated with an increased risk of developing endometrial carcinoma (Potischman *et al.* 1996, Kaaks *et al.* 2002, Lukanova *et al.* 2004, Allen *et al.* 2008, Audet-Walsh *et al.* 2011). Potischman and coworkers also reported that high circulating levels of A4 were associated with 2.8-fold increased risks of developing endometrial cancer among postmenopausal women, following the adjustment for other factors (Potischman *et al.* 1996). These authors subsequently concluded that circulating levels of A4 yielded most significant and consistent correlation with the risks of developing endometrial carcinoma compared with those of estrone (E_1), estradiol (E_2) and E_1-sulfate (E_1S) (Potischman *et al.* 1996). Kaaks and coworkers also reviewed numerous epidemiological studies and concluded that endometrial carcinoma risks were increased among pre- and postmenopausal women associated with elevated plasma A4 and testosterone (Kaaks *et al.* 2002). Thereafter, two prospective studies have reported discrepant results for the association of cancer risk with serum androgen levels. A multicenter prospective study also demonstrated that elevated circulating levels of A4 and DHEAS were positively associated with endometrial cancer risk, although free testosterone did not correlate with an increased risk among postmenopausal women (Lukanova *et al.* 2004). In contrast, elevated circulating levels of total and free testosterone were positively associated with endometrial cancer risk, although A4 and DHEAS were not necessarily correlated with increased risks among postmenopausal women in the European Prospective Investigation into Cancer and Nutrition Study (EPIC) (Allen *et al.* 2008). Recently, using highly specific and sensitive validated methods based on liquid chromatography/electrospray tandem mass spectrometry (LC-MS/MS), Audet-Walsh and coworkers compared the levels of 18 circulating steroid hormones in 126 postmenopausal cases of endometrial carcinoma with 110 cases of healthy postmenopausal women and to investigate how these hormonal levels could be possibly related to clinical characteristics (Audet-Walsh *et al.* 2011). Most hormones, including DHEA, DHEAS, A4, testosterone and DHT, were significantly elevated in endometrial carcinoma cases compared with healthy controls. These particular epidemiological findings clearly indicate that elevated serum androgen levels increase the risks of developing endometrial carcinoma at least in postmenopausal women.

The impact of polycystic ovary syndrome (PCOS), a well-known common endocrine disorder affecting 5–8% of women with reproductive age and associated with menstrual irregularity, elevated androgen and polycystic ovaries (Azziz *et al.* 2004), has also been examined in relation to endometrial cancer risk,

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and given its association with increased androgenicity may give important clues to the link between androgens and this malignancy. A recent systemic review and meta-analysis revealed that women with PCOS have significant increased risks of endometrial carcinoma (odds ratio 2.79) compared with those without PCOS. In addition, the risks of women with PCOS increased further for endometrial carcinoma (odds ratio 4.05) less than 54 years of age (Barry *et al.* 2014). PCOS and endometrial carcinoma shared many of the similar risk factors (obesity, diabetes, anovulation, inflammation etc.), but elevated androgens themselves may be considered the pivotal factor for endometrial carcinoma risk. However, data on serum levels of androgen and risks of developing endometrial carcinoma among premenopausal women are yet to be completely established. To the best of our knowledge, only two prospective studies have been reported to date (Allen *et al.* 2008, Clendenen *et al.* 2016). Clendenen and coworkers recently reported that no significant association was detected between circulating androgens and risks of developing endometrial carcinoma before menopause, which was consistent with the results of other prospective study (Clendenen *et al.* 2016). However, it is entirely true that androgen concentrations obtained in these studies were measured by classical radioimmunoassay (Allen *et al.* 2008, Clendenen *et al.* 2016) with the inherent limitations involved in this methodology. Thus while these studies provide important preliminary evidence, future studies employing gold standard techniques of steroid assays would be ideal in resolving the question of circulating androgens and risks of developing endometrial carcinoma in premenopausal women.

Despite the information above suggesting a relationship between androgens and endometrial cancer, risk studies directly examining the effects of androgens on endometrial cancer cells have had mixed results in contrast to the consistent stimulation of proliferation observed with estrogens in the same models (Tuckerman *et al.* 2000, Braunstein 2007). In addition, in a more physiological setting, short-term exogenous testosterone treatment of postmenopausal women did not stimulate endometrial proliferation and counteract the proliferative effects induced by E_2 (Zang *et al.* 2007, Shufelt & Braunstein 2009). Therefore, the biological link between the increased endometrial carcinoma risks associated with elevated levels of androgen in circulation and androgen actions on endometrial cells is not well characterized or understood. Several investigators reported that elevated plasma levels of androgens resulted in increased tissue estrogen levels through their aromatization in peripheral or local tissues, including adipose, breast and endometrial carcinoma tissues (Sasano & Harada 1998, Bélanger *et al.* 2002, Somboonporn *et al.* 2004). Therefore, androgens may be locally converted into estrogens by adipose tissue and/or hiding small neoplastic cells and neoplastic cells could possibly grow rapidly in the presence of locally produced estrogens especially in postmenopausal women. Estrogen-dependent neoplasms such as breast and endometrioid endometrial carcinoma, in which *in situ* conversions from serum androgens to biologically active estrogens occur and are biologically important, are therefore considered as 'intracrine'-dependent malignancies.

In situ androgen metabolism and synthesis

Intracrinology

Recently, a great deal of focus has been given to the importance of intratumoral metabolism and synthesis of biologically active steroids in various systems. This is attributable to the documented importance of the interactions of various enzymes in the pathogenesis and progression of various human hormone-dependent neoplasms, including breast and endometrial carcinoma. This complex system, shown in a simplified version in figure one, means that enormous local control over the types and amounts of steroids produces and specific to one tissue is possible. Results of numerous studies have demonstrated increased tissue estrogen content in human breasts, compared with serum and/or normal non-neoplastic tissues of the same patients (van Landeghem *et al.* 1985, Pasqualini & Chetrite 1999, Chetrite *et al.* 2000). In these studies, the tissue concentrations of E_1 and E_2 were generally several times higher than those detected in the plasma or in the area of the normal breast tissues of the same postmenopausal patients, despite markedly low levels of circulating estrogens. In contrast to breast carcinoma cases, there is limited data regarding tissue estrogen concentrations in endometrial carcinoma tissues (Bonney *et al.* 1986, Vermeulen-Meiners *et al.* 1986, Naitoh *et al.* 1989, Berstein *et al.* 2003). Berstein and coworkers examined 78 endometrial carcinomas and detected higher concentrations of E_2 in carcinoma tissue specimens compared with macroscopically normal endometrium (Berstein *et al.* 2003). Our findings (Ito 2005, Ito *et al* 2006) were generally consistent with those of other studies demonstrating higher E_2 levels associated with carcinoma. These results suggest that intratumoral estrogen metabolism and synthesis are pivotal in the progression of endometrial carcinoma, although the available data of sex-steroid hormone concentrations were all obtained by classical radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) and not by the modern gold standard methods of mass spectrometry. This distinction may be important as the classical methods of RIA and ELISA have been the standard methodology to analyze steroid hormones; however, these methods harbor several problems, including sensitivity and specificity/cross-reactions (Rauh 2012). On the other hand, LC-MS/MS is highly specific and sensitive to validated methods, which could measure highly specific hormones from very small amounts of the sample (Rauh 2012).

The status of intratumoral androgen concentration has been less frequently explored than that of estrogen in breast and endometrial carcinoma. Among the androgens, DHT binds with the highest affinity to androgen receptor (AR), and together with testosterone promotes AR transcriptional activity, while A4 and DHEA have extremely low binding affinity for AR (Avancès *et al.* 2001). Potent androgen DHT concentrations were significantly higher in breast carcinoma tissues than in plasma (Recchione *et al.* 1995), and the tissue concentration of DHT was three-fold higher in ductal carcinoma *in situ* of the breast compared with the non-neoplastic breast (Shibuya *et al.* 2008). The analysis of serum DHT concentration in the patients of endometrial carcinoma was reported (Audet-Walsh *et al.* 2011), but not in the cases of DHT concentration in endometrial tumor tissues. We recently measured the tissue and serum concentrations of testosterone and DHT in 31 endometrioid endometrial adenocarcinoma tissue and 5 normal endometrium samples using LC-MS/MS methods (Tanaka *et al.* 2015) with the advantages mentioned above. This study revealed that a markedly elevated DHT tissues/serum concentration ratio was detected in endometrioid endometrial adenocarcinoma tissues compared with that in normal endometrial tissues (8.0-fold). This finding also indicated that local production of DHT in endometrioid endometrial adenocarcinoma could be similar to that of breast carcinoma. Among the enzyme system required for local biosynthesis of androgen, 17β-hydroxysteroid dehydrogenase type 5 (17β-HSD5: conversion from A4 to testosterone) and 5α-reductase (5α-Red: reduction of testosterone to DHT) are the principal enzymes involved in the formation of biologically active androgen, DHT. The examination of these responsible enzymes in endometrial carcinoma tissues is very important to obtain the better understanding of the significance of androgen in endometrial carcinomas.

17β-HSD5

17β-HSDs are key enzymes involved in the formation of biological active sex steroids, including testosterone, E_1 and E_2. The reduction of A4 is known to result in the production of testosterone mainly by 17β-HSD5 (Dufort *et al.* 1999). 17β-HSD5, a reductive 17β-HSD, is a member of aldo-keto reductase superfamily and formally termed AKR1C3, whereas the others are members of the short-chain alcohol dehydrogenase (Deyashiki *et al.* 1995). 17β-HSD5 is expressed in various peripheral tissue, liver, prostate, ovary and has been also reported in prostate and breast carcinoma tissues (Luu-The *et al.* 2001, Vihko *et al.* 2004, Suzuki *et al.* 2010).

Increased 17β-HSD5 mRNA and protein expression were reported in endometrial carcinoma tissues compared with normal endometrium (Rižner *et al.* 2006, Smuc & Rizner 2009). However, one group reported weaker protein expression of 17β-HSD5 in hyperplastic and cancerous endometrium compared with normal proliferative endometrium (Zakharov *et al.* 2010) and no significant difference of mRNA expression between cancerous and normal endometrial tissues was detected in other groups (Cornel *et al.* 2012, Sinreih *et al.* 2013). In our previous study, 17β-HSD5 immunoreactivity was detected in 18/36 (50%) and 71/103 (69%) cases of endometrial hyperplasia and endometrioid endometrial carcinoma, respectively. However, 17β-HSD5 immnoreactivity was detected only in 5/26 (19%) and 5/20 (25%) cases of proliferative and secretory phase endometria, respectively (Ito 2005, Ito *et al.* 2006). 17β-HSD5 immnoreactivity in endometrioid endometrial carcinoma was significantly higher than that of proliferative and secretory phase and endometrial hyperplasia. Due to the inconsistent and limited data on the available studies of 17β-HSD5 expression, further investigation is warranted.

5α-reductase

5α-reductase (5α-Red) is an enzyme responsible for the conversion of testosterone to a most potent androgen, DHT. This enzyme is considered to be an important regulator of local actions of androgens. Two 5α-Red isoenzymes, Types 1 and 2 (e.g. 5α-Red1 and 5α-Red2), have been cloned and characterized in humans (Russell & Wilson 1994). In 18 normal cycling human endometria, 5α-Red1 and 5α-Red2 were both detected in the cytoplasm of epithelial cells, with weak or focal perinuclear immunolocalization patterns through all phases of the menstrual cycle (Ito *et al.* 2002). 5α-Reds mRNA and protein were also

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detected in biopsies of pelvic endometriosis, as well as in the eutopic endometrium (Carneiro *et al.* 2008). In our previous study, immunoreactivity of 5α-Red1 and 5α-Red2 was detected in 37/44 (84%) and 34/44 (77%) cases of endometrioid endometrial carcinoma, respectively (Ito *et al.* 2002). 5α-Reds protein expression was demonstrated in the cytoplasm of carcinoma cells. Endometrial carcinoma tissue homogenates were associated with mRNA expression in the great majority of the cases examined. However, mRNA expression of 5α-Red2 was significantly down-regulated and that of 5α-Red1 was unchanged in endometrial carcinoma versus adjacent control endometrium, including proliferative, secretory and menopausal status (Sinreih *et al.* 2013). Recently, we measured the tissue concentration of androgen and performed immunohistochemical analyses of AR and 5α-Reds in 36 endometrioid endometrial carcinoma cases (Tanaka *et al.* 2015). Results of our study did reveal that 5α-Red1 immunoreactivity was detected in approximately 64% of endometrioid endometrial carcinoma and was positively correlated to intratumoral DHT concentration with statistical significance. No significant correlation was observed between 5α-Red2 immunoreactivity and the intratumoral concentration of DHT, although the immunoreactivity of 5α-Red2 was similar to that of 5α-Red1. In breast carcinoma, which has estrogen-dependent growth similar with endometrial carcinoma, DHT is considered to be locally produced mainly *via* 5α-Red1, because intratumoral DHT concentration was positively associated with 5α-Red1 (Suzuki *et al.* 2007). Therefore, 5α-Red1 is reasonably postulated to act predominantly for the local production of DHT in endometrial carcinoma, as reported in breast carcinoma.

Negative regulator for *in situ* DHT production in endometrial cancer

A4 and testosterone are precursors of not only DHT production, but also those of estradiol synthesis, as the substrate of aromatase (Fig. 1). Aromatase is an enzyme which catalyzes the conversion for androgens, mainly A4 and testosterone, to E_1 and E_2, respectively (Bulun *et al.* 2005). DHT itself is nonaromatizable. Therefore, aromatase could be a negative regulator for *in situ* production of DHT in endometrial carcinoma tissues by possibly reducing concentration or availability of the precursor testosterone and/or A4. Of particular interest, in breast carcinoma, which has estrogen-dependent growth similar to endometrioid endometrial carcinoma, intratumoral concentration of DHT was significantly correlated with that of testosterone (Recchione *et al.* 1995), and aromatase expression was inversely correlated with intratumoral DHT concentration (Suzuki *et al.* 2007) suggesting the functional possibilities of such a hypothesis and its potential relevance in endometrial carcinoma.

We previously demonstrated marked aromatase immunoreactivity and mRNA, mainly in the stromal cells of endometrioid endometrial carcinoma, but not in normal or hyperplastic endometrium (Watanabe *et al.* 1995). This result suggested an induction of aromatase

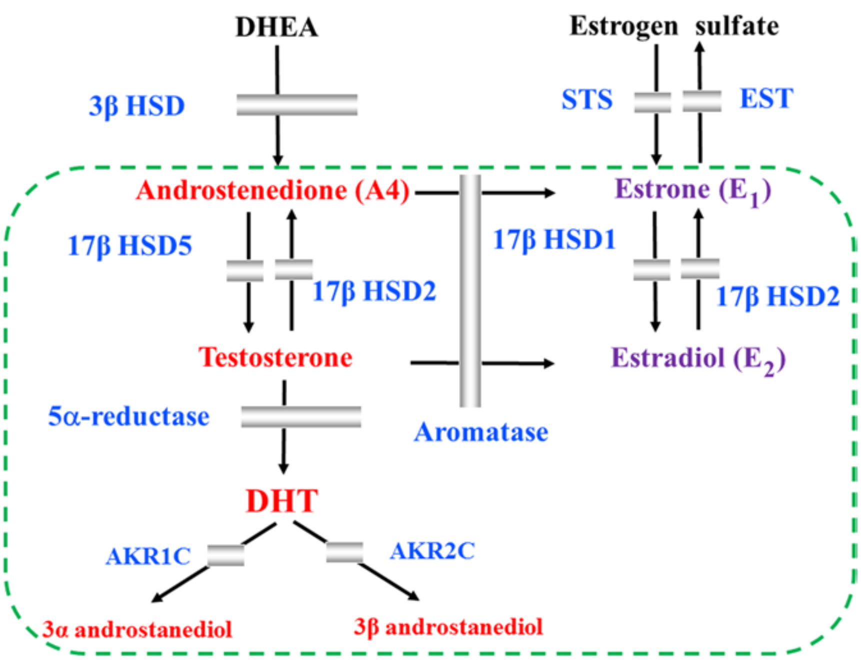

Figure 1
Schema showing production and metabolism of androgens in human tissues. 17βHSD, 17β-hydroxysteroid dehydrogenase; AKR1C, 3α-keto-reductase; AKR2C, 3β-keto-reductase; DHEA, dehydroepiandrosterone; DHT, 5α-dihydrotestosterone; EST, estrone sulfotransferase; STS, steroid sulfatase.

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expression by tumor–stromal interactions. In addition, a significant correlation was detected between aromatase expression in stromal cells and poor prognosis in the patients with endometrial carcinoma (Segawa *et al.* 2005), although several studies reported that aromatase expression was demonstrated in carcinoma and stromal cells together (Segawa *et al.* 2005, Jongen *et al.* 2009, Che *et al.* 2014). It is very important to consider *in vitro* stromal–carcinoma cell interactions when studying aromatase expression *in vitro*. We previously examined whether estrogen biosynthesis in the tumor microenvironment promoted endometrial carcinoma or not. In order to study the contribution of stromal cells to estrogen signaling pathways in endometrial carcinoma, we examined reporter cells stably transfected with the estrogen response element (ERE) fused to the destabilized green fluorescent protein (GFP) gene (Matsumoto *et al.* 2008). In this system, the ERE of carcinoma cells was activated to a variable extent by intratumoral stromal cells isolated from endometrial carcinoma. When testosterone was added as a substrate for aromatase, the GFP expression levels increased. Thereafter, these effects were variably inhibited by aromatase inhibition. These results indicated that GFP expression is driven by estrogen synthesized by aromatase in the endometrial cancer stromal cells. In addition, in order to further confirm the local biosynthesis of estrogens and tumor–stromal interactions on aromatase activity, endometrial carcinoma cell lines were cocultured with stromal cells isolated from endometrial carcinomas, and aromatization activity was measured using LC-MS/MS (Takahashi-Shiga *et al.* 2009). We also examined the effects of aromatase inhibitors on cell proliferation. Aromatase activity was significantly higher in the coculture with carcinoma cell lines and stromal cells than in each monoculture, respectively. Cell proliferation was significantly inhibited in carcinoma cells treated with aromatase inhibitors compared with control cultures. Recently, Che and coworkers also reported that aromatase expression in stromal, but not in carcinoma epithelium cells, was associated with interleukin 6 expression in epithelial cells of carcinomas by immunohistochemistry, which were subsequently confirmed also using laser capture microdissection/real-time RT-PCR in endometrial carcinoma tissues (Che *et al.* 2014). Their results did demonstrate the presence of the activation of a positive feedback loop in which interleukin 6 stimulated by E_2 in endometrial carcinoma epithelial cells induced aromatase expression in stromal cells, thereby promoting intratumoral E_2 synthesis. These findings above did confirm the importance of carcinoma–stromal cells interaction in the process of induction of aromatase in endometrial carcinoma tissues, and demonstrated that aromatase is a key enzyme in the local biosynthesis of estrogen in endometrial carcinoma as well as breast carcinoma. However, further investigation should be required to clarify the association between the expression of aromatase and intratumoral DHT concentration in endometrial carcinoma.

DHT is catalyzed to 3α and 3β androstanediol, which are relatively inactive, by 3α-keto-reductase (AKR1C) and 3β-keto-reductase (AKR1C2), respectively. The enzyme of AKR1C2 has the predominant catalytic efficiency. Rižner & Penning (2014) reviewed the possible roles of AKR1 enzymes in human steroid metabolism and alluded on the possibility of AKR1C gene expression in endometrial carcinoma. However, AKR1C1 and AKR1C2 mRNA expression in cancerous endometrium did not differ significantly to that in adjacent control normal tissue (Smuc & Rizner 2009, Sinreih *et al.* 2013). Therefore, it is unlikely that AKR1C1 and AKR1C2 serve as negative regulators of *in situ* production of DHT in endometrial carcinoma.

Androgen receptor and androgen action in endometrial cancer

Structure of androgen receptor

Androgens mediate their effects through the androgen receptor (AR). AR belongs to the nuclear receptor subfamily 3 group C gene 4 (NR3C4) and also to the nuclear receptor superfamily for steroid hormones. DHT binds with the highest affinity to AR, and together with testosterone, promotes AR transcriptional activities (Avancès *et al.* 2001). A polymorphic CAG repeat in exon 1 of the AR gene is well known to encode a polyglutamine tract and to increase the length of CAG repeat, which is also well known to be associated with decreased transactivation activities (Chamberlain *et al.* 1994). Of particular interest, a statistically significant inverse association has been reported between increased CAG repeat length and endometrial carcinoma risk in case-control studies (McGrath *et al.* 2006, Ashton *et al.* 2010), although controversies exist (Yang *et al.* 2009). On the other hand, Yang and coworkers evaluated 36 sex hormone-related genes using a tagging approach in a population-based case-control study of 417 endometrial carcinoma cases and reported that the possible evidence of the correlation with endometrial cancer risk could be the common genetic variation in

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AR (Yang *et al.* 2010). All of these results above did suggest that variant structures of AR played pivotal roles in the risks of developing endometrial carcinoma.

Androgen receptor expression

In the nonmalignant endometrium, AR was reported to be expressed in the endometrium throughout the menstrual cycle (Mertens *et al.* 2001, Apparao *et al.* 2002, Ito *et al.* 2002, Marshall *et al.* 2011, Gibson *et al.* 2014). The status of AR immunoreactivity in stromal cells is higher than that of epithelial AR throughout the menstrual cycle (Apparao *et al.* 2002). In our previous study, AR immunoreactivity was detected in the nuclei of both epithelial and stromal cells (Ito *et al.* 2002). AR immunoreactivity was also detected in the nuclei of 60–70% of stromal cells in the proliferative phase, while negative at functional is of endometrium in the secretory phase. AR immunoreactivity was detected in the nuclei of around 5–15% of epithelial cells in the secretory phase, while negative or very weak in the proliferative phase. Endogenous and exogenous androgens should have the ability to alter endometrial function through AR expression. For example, in women with PCOS, who generally exhibit chronic hyperandrogenism, exhibited elevated endometrial AR immunoexpression has been documented compared with normal fertile control (Apparao *et al.* 2002) suggesting the effects on androgens in changing the intracrine environment of these tissues. In a similar vein, albeit circumstantial, correlation between endometrial AR expression and serum androgens levels has also been demonstrated in the context of obesity and weight loss. Serum androgens are reported to be positively correlated with BMI (Allen *et al.* 2008) and abdominal fat accumulation (Cao *et al.* 2013), but in asymptomatic morbidly obese women in response to interventions that reduce abdominal fat and/or BMI such as bariatric surgery and weight loss, AR immunoexpression dropped significantly in response to these interventions (Argenta *et al.* 2014) perhaps hinting at the correlation between serum androgens and endometrial AR expression.

The expression of AR in endometrial carcinoma has been detected in several studies (Horie *et al.* 1992, Sasaki *et al.* 2000, Ito *et al.* 2002, Qiu *et al.* 2014, Tanaka *et al.* 2015). The number of cases was small ($n=4$), but Horie and coworkers reported that all specimens of endometrial carcinoma tissues examined expressed AR (Horie *et al.* 1992). On the other hand, Sasaki and coworkers reported that AR was not detected in 79% of endometrial carcinomas, although their cases included several unknown histological types (Sasaki *et al.* 2000). Qiu and coworkers recently reported AR-highly expressing cells in 50 of 76 cases (66%) of endometrial carcinoma (Qiu *et al.* 2014). In our previous study, AR immunoreactivity was detected in the nuclei of carcinoma cells and the number of positive cases was 39/44 (89%) (Ito *et al.* 2002). In the great majority of cases, carcinoma tissue homogenates did also have mRNA expression. Thereafter, AR expression was recently reported in 92 of 122 cases (82%) of endometrioid endometrial carcinoma (Tanaka *et al.* 2015). These studies all demonstrated that AR was expressed in the great majority of endometrial carcinoma, especially in endometrioid type.

Androgen action

The possible impact of androgen actions and the pivotal roles of androgen signaling through its receptor in endometrial carcinoma have not yet been clarified. DHT is nonaromatizable androgen and not converted to estrogen. DHT acts most markedly as an androgen through the AR pathway. Hackenberg and coworkers and Hackenberg & Schulz first demonstrated the inhibition of cell proliferation in human endometrial carcinoma cell line, MFE-296 cells, by DHT (Hackenberg *et al.* 1994, Hackenberg & Schulz 1996). Lovely and coworkers subsequently reported that DHT can act as antiestrogens through inhibiting the alkaline-phosphatase enzymatic activity in a dose-dependent manner, using hormonally responsive cell lines (a well-differentiated endometrial adenocarcinoma cell lines, Ishikawa) (Lovely *et al.* 2000). AR protein expression in Ishikawa cell lines was subsequently reported to be up-regulated by DHT or E_2 and to be inhibited by the antiandrogen, hydroxyflutamide or bicalutamide, or medroxyprogesterone acetate (MPA) based on Western blot analysis (Apparao *et al.* 2002). These studies consistently demonstrated that androgens had the important ability to inhibit cell proliferation through DHT-AR pathway in endometrial carcinomas *in vitro*. In contrast, *in vivo* animal model study demonstrated that DHT excess neither significantly suppressed nor increased the growth rate of well-differentiated human endometrial carcinoma implants in the nude mice, although AR expression in tumor tissues were not examined (Legro *et al.* 2001).

In breast carcinoma, DHT is considered to be locally produced mainly *via* 5α-Red1. In addition, immunohistochemical analysis showed that patients with positive for both AR/5α-Red1 demonstrated significant correlations with decreased risk of recurrence

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and improved prognosis for overall survival, and AR/5α-Red1 status was an independent prognostic factor (Suzuki *et al*. 2007). AR/5α-Red1 double-negative status was significantly correlated with worse prognosis even in triple-negative invasive breast carcinoma, which is defined as the absence of ER, PR and human epidermal growth factor receptor 2 (HER2) expression in tumor cells (McNamara *et al.* 2013). In addition, the absence of intratumoral androgenic enzymes, both 5α-Red1 and 17β-HSD5, was significantly associated with adverse clinical outcome in patients with invasive lobular carcinoma of the breast (Yoda *et al.* 2014). All of these findings above did indicate that DHT could inhibit the cell proliferation through DHT-AR pathway and influence the eventual clinical prognosis of the patients with breast cancer.

Using the logic and evidence of studies in the breast as guidelines, it then becomes clear that it is very important to analyze the correlation between the status of AR and 5α-Reds and clinicopathological findings to further clarify the clinical significance of DHT-AR pathway in endometrial carcinoma. AR expression was significantly inactivated by hypermethylation of the AR gene CpG islands in human endometrial carcinoma compared with normal endometria, using pairs of carcinomatous and normal samples from 28 patients (Sasaki *et al.* 2000). All carcinoma samples from advanced stage did demonstrate only methylated AR alleles and immunoexpression of AR negative, although about half of the cases from early stage harbored these alleles. In addition, Rodríguez and coworkers had genotyped both CAG and GGN repeats from 204 cases of endometrial carcinoma tissues and revealed that the presence of short CAG or GGN repeats of the AR genes in a tumor specimens was correlated with a favorable conditions of prognostic values in endometrial carcinoma (Rodríguez *et al.* 2006). Shortening each repeat has been considered to increase either the amount of AR protein (GGN) or its activity (CAG) (Chamberlain *et al.* 1994, Ding *et al.* 2005). In our recent study using 86 endometrioid endometrial adenocarcinoma samples with immunohistochemical analysis, the AR-positive status was significantly correlated with progression-free survival (PFS), but not with endometrial carcinoma-specific survival (ECSS) in endometrial carcinoma patients (Tanaka *et al.* 2015). 5α-Red1-positive status was significantly associated with both PFS and ECSS in those patients. In addition, AR/5α-Red1 double-negative status was significantly correlated with worse PFS and ECSS, when these patients were divided into four groups, according to the status of AR and 5α-Red1, such as AR/5α-Red1 double positive, positive/negative, negative/positive and double negative status, respectively (Tanaka *et al.* 2015). In addition, Kamal and coworkers recently reported that AR was expressed in postmenopausal endometrial epithelium and their subsequent loss in endometrial carcinoma was significantly associated with disease-free survival (Kamal *et al.* 2016). Those results consistently demonstrated that a lack of androgen action in intratumoral levels is correlated with a poor prognosis in the patients, and androgen signaling exerts anticancer effects through DHT-AR pathway in endometrial carcinoma. Results of our study also indicated that it was important to evaluate both AR status and 5α-Red1, the latter serving as a surrogate marker of intratumoral DHT production, for understanding the effect of androgenic signaling on carcinoma progression (Miki *et al.* 2015, Tanaka *et al.* 2015).

Androgen action from the viewpoint of therapeutic target

In order to understand the anticancer effects through DHT-AR pathway, it is necessary to analyze the downstream signals of AR. However, such DHT-induced genes have remained largely unknown in endometrial carcinoma. Qiu and coworkers recently demonstrated that the mRNA expression of AR target gene (XBP1, MYC, ZBTB16, and UHRF1) increased after treatment with DHT in the human endometrial carcinoma cell line MFE-296 cells, although the roles of those target genes has still remained virtually unknown (Qiu *et al.* 2014). In addition, the possible roles of androgen-induced genes in endometrial carcinoma have been recently elucidated. In breast carcinoma cells, DHT predominantly exerted antiproliferative effects on mitogenic effects of estrogens (Lapointe & Labrie 2001). Those inhibitory effects were correlated with increased levels of cyclin-dependent kinase inhibitor p21 and/or p27 (Lapointe & Labrie 2001, Greeve *et al.* 2004). Takagi and coworkers also reported that E_2-mediated proliferation was significantly inhibited by DHT in T-47D breast carcinoma cells, which express AR and ER (Takagi *et al.* 2010). This proliferation was also associated with an increase in 17β-HSD2 expression level and 17β-HSD2 was induced by DHT in a dose-dependent manner. In addition, local E_2 concentration was inversely associated with 17β-HSD2 status in breast carcinoma tissues. It is well known that 17β-HSD2 preferentially catalyzes testosterone and E_2, to A4 and E_1, respectively (Wu *et al.* 1993). However, 17β-HSD2 expression was reported to be significantly associated with decreased risk to develop late relapse of breast carcinoma (Gunnarsson *et al.* 2001, 2005).

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In addition, significant positive association was reported between the status of androgenic enzymes, 17β-HSD5 and 5αR1, and 17β-HSD2, and in particular, 17β-HSD2 expression was inversely correlated with tumor size in invasive lobular carcinoma of the breast (Yoda *et al.* 2014). These findings above all indicated that DHT acted, at least partly, through the up-regulation of 17β-HSD2 and decreasing local E_2 concentration, and finally played the pivotal roles involved in antiproliferative effects in breast cancer. We have recently reported that 17β-HSD2 immunoreactivity was detected in 37% of endometrial carcinoma cases and correlated with 17β-HSD2 enzymatic activity and expression of 17β-HSD2 mRNA (Utsunomiya *et al.* 2001). In normal endometrium, 17β-HSD2 immunoreactivity was present at all the cases of secretory phase, but not at any endometrial mucosa of proliferative phase. 17β-HSD2 mRNA expression was consistently detected in endometrial carcinoma compared with adjacent normal endometrium at menopausal status in several studies (Lépine *et al.* 2010, Cornel *et al.* 2012, Sinreih *et al.* 2013). Therefore, the enzyme of 17β-HSD2 should play an important role to modify the balance of estrogen production in not only breast but also endometrial carcinoma. If DHT is involved in the modulation of *in situ* estrogen metabolism by stimulating the expression of 17β-HSD2, DHT could be one of the important candidates as a new endocrine-related agent in endometrial carcinoma. Further investigation should be required to clarify the therapeutic roles of DHT in endometrial carcinoma.

As we mentioned above, aromatase is a negative regulator for *in situ* production of DHT. Aromatase-inhibitory therapeutic agents are considered the most effective endocrine therapies in postmenopausal patients with estrogen-dependent breast carcinoma (Bulun *et al.* 2005). Of particular interest, intratumoral concentration of DHT in breast carcinoma tissue was significantly higher in the patients treated with exemestane, the aromatase inhibitor, than in those who did not receive this therapy (Takagi *et al.* 2010). This result indicated that aromatase-inhibitory agents could cause increased concentrations and actions of DHT, in conjunction with estrogen deprivation in breast carcinoma. In contrast, there remain some controversies as to whether or not aromatase-inhibitory agents could be effective in the patients with endometrial carcinoma. Previous Gynecologic Oncology Group Study (GOG) could not possibly demonstrate distinct clinical efficacy with aromatase inhibitor treatment (Rose *et al.* 2000). Thereafter, a prospective phase II trial for fulvestrant was conducted in 53 recurrent or persistent endometrioid endometrial carcinoma (Covens *et al.* 2011). Patients were stratified into subsets of ER-positive and -negative patients. In ER-positive patients, 3, 13 and 29% cases demonstrated a complete or partial response and stable disease, although no cases were associated with either a complete or a partial response, and 18% demonstrated stable disease in ER-negative patients. Nordic Society of Gynecologic Oncology Study (NSGO) reported similar results (Lindemann *et al.* 2014). Thangavelu and coworkers examined the biological changes in human endometrial carcinoma tissues before and after aromatase inhibitor treatment (Thangavelu *et al.* 2013). Patients were randomized to receive anastrozole or placebo before definitive surgery. Treatment with anastrozole caused a marked decrement in proliferation as demonstrated by decreased Ki-67 expression. Sasano previously reported similar results using [^{3}H] thymidine uptake or Ki-67 labeling (Sasano *et al.* 1999). Therefore, it is important to clarify the roles, including the possibility of application and indication, of aromatase-inhibitory drugs in hormone-receptor positive or hormone-sensitive endometrial carcinoma in postmenopausal patients. In addition, aromatase inhibitor therapy might be established as one form of endocrine treatment of endometrial carcinoma, if aromatase-inhibitory drugs cause not only estrogen deprivation but also increased antiproliferative actions of DHT. Further analysis is required to clarify the clinical importance of DHT actions in association with the therapeutic efficacy of aromatase-inhibitory drugs in endometrial carcinoma.

Summary and conclusion

Local androgens exert both direct and indirect impacts on endometrial carcinoma. Testosterone is not only a precursor of estrogen synthesis as the substrate of aromatase, but also a precursor of DHT production as the substrate of 5α-Red enzymes. Recent studies revealed that *in situ* DHT synthesis occurred at the same time with estrogen synthesis, and DHT signaling could directly exert anticancer effects through DHT-AR pathway in endometrial carcinoma. Aromatase is a negative regulator for *in situ* production of DHT. If aromatase-inhibitory agents cause not only estrogen deprivation but also increased antiproliferative actions of DHT, combination therapy of DHT and aromatase inhibitor agents could be one of the important candidates as a new endocrine-related agent for endometrial carcinoma especially in postmenopausal patients. However, the downstream signals of AR, which are directly involved in anticancer effects, have remained largely unknown in endometrial carcinoma. Further investigation should be required to understand the mechanisms and impacts of anticancer signaling by AR-activation for establishing the possible androgen therapies.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement
K I drafted the manuscript. Y M, T S, K M M and H S edited the manuscript. All authors read and approved the final manuscript.

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