Evidence of androgen action in endometrial and ovarian cancers

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Abstract

Endometrial cancer (EC) and ovarian cancer are common gynaecological malignancies. The impact of androgen action in these cancers is poorly understood; however, there is emerging evidence to suggest that targeting androgen signalling may be of therapeutic benefit. Epidemiological evidence suggests that there is an increased risk of EC associated with exposure to elevated levels of androgens, and genetic variants in genes related to both androgen biosynthesis and action are associated with an increased risk of both EC and ovarian cancer. Androgen receptors (ARs) may be a potential therapeutic target in EC due to reported anti-proliferative activities of androgens. By contrast, androgens may promote growth of some ovarian cancers and anti-androgen therapy has been proposed. Introduction of new therapies targeting ARs expressed in EC or ovarian cancer will require a much greater understanding of the impacts of cell context-specific AR-dependent signalling and how ARs can crosstalk with other steroid receptors during progression of disease. This review considers the evidence that androgens may be important in the aetiology of EC and ovarian cancer with discussion of evidence for androgen action in normal and malignant endometrial and ovarian tissue.

Key Words

- ▶ androgen
- ▶ androgen receptor
- ▶ carcinoma
- ▶ endometrium
- ▶ ovary

Endocrine-Related Cancer
(2014) 21, T203–T218

Introduction

Endometrial cancer (EC) and ovarian cancer are common gynaecological malignancies; however, the impact of androgen action in these cancers is poorly understood. Epidemiological evidence suggests that androgens may be important in the aetiologies of EC and ovarian cancer. Androgen receptor (AR) expression has been described in these malignancies and androgen action has been proposed as a potential therapeutic target. Evidence from cell and animal studies suggests that androgens may have anti-proliferative actions in EC. By contrast, the impact of androgens in ovarian cancer is debated, but some studies suggest that androgens may stimulate ovarian epithelial cell proliferation.

In both normal and malignant tissues, androgen action in the female reproductive system requires a

complex convergence of sex steroid biosynthesis, local metabolism and receptor activation. Alterations in any of these elements could have an impact on risk or progression of malignancy. This review appraises the evidence that androgens may be important in the aetiology of EC and ovarian cancer with discussion of disease mechanisms and treatment options.

Epidemiology of EC and ovarian cancer and risks associated with androgen exposure

Endometrial cancer

EC is the most common gynaecological cancer and the fourth most common cancer in women in the UK

(<http://www.cancerresearchuk.org>). Clinically, ECs are graded according to the FIGO system (revised in 2009 (Lewin 2011)) and have classically been divided into two subtypes: oestrogen-dependent type I and the less common, but clinically more aggressive, oestrogen-independent type II (Emons *et al.* 2000). Approximately 95% of endometrial tumours are adenocarcinomas arising due to malignant transformation of the endometrial glandular epithelium (<http://www.cancerresearchuk.org>). An integrated molecular classification of ECs was recently described combining data from genomic, transcriptomic and proteomic analyses of over 370 ECs leading to further classification of EC subtypes (The Cancer Genome Research Network, Kandoth *et al.* (2013)). Utilising array-based and sequencing technologies, ECs were classified into four major groups: i) ultramutated cancers with DNA polymerase epsilon (*POLE*) mutations (7%), ii) hypermutated cancers due to DNA mismatch repair and associated microsatellite instabilities (28%), iii) ECs with low mutation rate and a low frequency of DNA copy-number alterations (CNA, 39%) and iv) ECs with low mutation rate but high-frequency DNA CNA (26%). The cancers in the first three groups were almost all endometrioid carcinomas (type I). The fourth group included uterine serous carcinomas and serous-like or serous-related carcinomas thought not to be related to oestrogen exposure or obesity (type II). Although type II ECs are classified as oestrogen-independent, new evidence suggests that both types I and II ECs share many common risk factors, including age at menarche, parity and contraceptive use which may indicate that changes in steroid exposure has an impact on the risk of developing either type I or II EC (Setiawan *et al.* 2013). Using data collected in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, factor analysis of 233 cases of EC with 446 matched controls identified three factors associated with increased post-menopausal EC risk: 'insulin resistance', 'steroids' and 'inflammation' (Dossus *et al.* 2013).

The greatest incidence of EC occurs between the ages of 50 and 65 years with peak incidence occurring after menopause (Purdie & Green 2001). Established risk factors for developing EC are associated with increased exposure to oestrogens throughout the reproductive lifespan such as nulliparity, early menarche and late menopause (Purdie & Green 2001). Risk is also associated with exposure to selective oestrogen receptor modulators (SERMs) such as tamoxifen administered as part of treatment for breast cancer (Bergman *et al.* 2000). Incidence of EC increased in the 1970s as a result of oestrogen-only hormone replacement therapy (HRT) use; however, these rates were reduced

following the inclusion of progestins in HRT formulations that significantly decrease risk of EC due to the anti-proliferative effects of progestins on the endometrium (Beral *et al.* 2005). Consistent with the anti-proliferative effect of progestins, use of the combined oral contraceptive pill is considered as protective against EC risk (Emons *et al.* 2000). While the role of progestins and 'unopposed oestrogen' exposure as a determinant of EC risk are well established, the effect of androgen action and exposure on EC risk is less well defined.

In a further analysis of data from the EPIC study, pre-diagnostic concentrations of endogenous hormones including testosterone, androstenedione (A4) and DHEAS were measured in pre- and post-menopausal women. Elevated circulating levels of free testosterone positively correlated with EC risk, but A4 and DHEAS were not associated with increased risk (Allen *et al.* 2008). The Million Women Study identified that BMI was a major modifiable risk factor and that as many as half of the EC cases in post-menopausal women could be attributed to being obese or overweight (Reeves *et al.* 2007). Furthermore, an analysis of EC cases in European women estimated that obesity accounted for 39% of cases (Bergstrom *et al.* 2001), an association usually attributed to excess exposure to oestrogens as adipose tissue is the primary site of oestrogen production in post-menopausal women (Simpson *et al.* 1997). Interestingly, a study from the 1980s by Samojlik *et al.* (1984) examined androgen production in morbidly obese, non-hirsute, normally menstruating women and found that testosterone and dihydrotestosterone (DHT) production rates were elevated twofold compared with controls, but that this increased androgen production was offset by enhanced metabolic clearance. Despite the enhanced clearance described by the authors, the enhanced serum concentrations of androgens suggest that obesity may be a hyperandrogenic state. Consistent with this, recent analyses in the EPIC study demonstrated that concentrations of free testosterone were positively correlated with BMI (Allen *et al.* 2008). Furthermore, free testosterone and DHEAS are reported to correlate positively with abdominal fat accumulation in post-menopausal women (Cao *et al.* 2013).

Polycystic ovarian syndrome (PCOS) is an endocrine disorder affecting women of reproductive age and is associated with menstrual cycle disturbances, hyperandrogenism and infertility (Hart *et al.* 2004). Exposure to elevated androgens during the reproductive years in women with PCOS may adversely impact on endometrial function. PCOS has long been associated with an increased risk of EC and prolonged periods of anovulation may

increase exposure to both androgens and oestrogens. PCOS and EC share many of the same risk factors and a recent population-based case-control study in Australia found that women with PCOS have a fourfold increased risk of EC compared with women without PCOS (Fearnley *et al.* 2010). This risk was elevated for type I cancers and had a greater association with symptoms of androgen excess such as hirsutism (OR 2.4, all EC cases) and irregular periods (OR 3.1, all EC cases) (Fearnley *et al.* 2010).

Ovarian cancer

According to a recent report from FIGO, ovarian cancer is the seventh most common cancer in women worldwide (Prat 2013). In the UK, it is the fifth most common female cancer (<http://www.cancerresearchuk.org/>) consistent with higher incidence in high-resource countries (Ferlay *et al.* 2010). The aetiology of ovarian cancer is not fully understood and work on predictive biomarkers has been hampered by failure to separate results on the basis of histological subtype (Sieh *et al.* 2013a). Approximately 90% of ovarian tumours are carcinomas (malignant epithelial tumours) with five main types being distinguished based on histopathology and molecular genetic analysis (Prat 2012, Tan *et al.* 2013). Although historically often treated as a single disease entity, new evidence has revealed that histologically similar ovarian cancers may differ in their tissues of origin, their genetic abnormalities and their responsiveness to chemotherapy (Tan *et al.* 2011). A recent GWAS study reported six loci that were associated with subtype-specific epithelial ovarian cancer risk (Earp *et al.* 2013) underlining the importance of redefining the origins of some subtypes. The Australian Ovarian Cancer Study group reported strikingly similar patterns of risk for serous ovarian and fallopian tube cancers and different results for primary peritoneal cancers suggesting that the former may be linked (Jordan *et al.* 2008). Additional studies have reported that the protective effect of tubal ligation is subtype specific with risk reduction for invasive endometrioid and clear cell cancers (Sieh *et al.* 2013b). Studies on women with *BRCA1/BRCA2* mutations have provided convincing evidence that high-grade serous cancers can arise from cells within the fallopian tube (reviewed in Berns & Bowtell (2012)).

Non-epithelial ovarian cancers include sex cord tumours, germ cell tumours, yolk sac tumours and ovarian-Leydig tumours – together they account for ~10% of ovarian cancers (Van Nieuwenhuysen *et al.* 2013). The majority are granulosa cell tumours (GCTs) with the adult form typically detected in women in their 1950s who

present with post-menopausal vaginal bleeding (Schumer & Cannistra 2003). Adult GCTs are thought to arise from granulosa cells in late pre-ovulatory follicles and similar to their pre-malignant counterparts they secrete steroids including oestrogen, and in rare cases, patients may present with evidence of hyperandrogenism (Vera *et al.* 2013).

As with other female cancers, the strongest risk factor for development of ovarian cancer is age. In the UK, between 2008 and 2010, 75% of cases were diagnosed in women aged 55 and over (<http://www.cancerresearchuk.org/>). A large Swedish study has reported that women who were admitted to hospital for ovarian cysts before the age of 29 were at increased risk of ovarian cancer later in life (Borgfeldt & Andolf 2004). Ovarian cancer risk is consistently reported as being reduced by factors that interrupt ovulation, including use of oral contraceptives, pregnancy or breastfeeding (Modugno *et al.* 2012) as well as tubal ligation with the latter most closely associated with endometrioid tumours (Rice *et al.* 2013). Pooled data from 12 prospective cohort studies in North America and Europe identified 2000 ovarian epithelial cancers in approximately half a million women. They reported an association between height >1.7 m and ovarian cancer risk especially in pre-menopausal women (Schouten *et al.* 2008). Gonadal hormones stimulate growth at puberty, hence an association between height and risk may be consistent with hormone action.

In their 2012 review of the epidemiological evidence that androgens might play a role in development of epithelial ovarian cancers, Modugno *et al.* argued that although some epidemiological evidence supported a role for androgens in development of disease, there were also studies that did not support this assertion (reviewed in Modugno *et al.* (2012)).

Results from the Million Women Study reported risks associated with use of HRT in post-menopausal women. Current users were significantly more likely to develop ovarian cancer than never users but in past users there was no elevation of risk. In current users, risks varied considerably according to cancer histology (Beral *et al.* 2007). Additional evidence for hormonal associations with risk may be inferred from the reported association between development of endometriosis, an oestrogen-dependent inflammatory disorder and ovarian cancer. Pooled analysis of 13 case-control studies found that endometriosis was associated with an increased risk of clear cell, low-grade serous and endometrioid invasive but not mucinous tumours (Pearce *et al.* 2012).

Many patients with symptoms of PCOS (see above) have elevated circulating concentrations of androgens that

may be associated with development of hirsutism (Franks 1995). Although some studies have reported increased risk of ovarian cancer in women with PCOS or hirsutism, results were based on very small numbers of patients. In a population-based Australia-wide study of ~1500 women with a new diagnosis of invasive epithelial ovarian cancer or borderline malignant tumour, there was no evidence that excess androgens due to PCOS or treatment with the synthetic androgen Danazol were correlated with overall risk of ovarian cancer but PCOS did increase the risk of borderline tumours (Olsen *et al.* 2008). However, in a small study of women taking Danazol as a treatment for endometriosis, Danazol use was associated with a 3.2-fold increased risk of ovarian cancer (Cottreau *et al.* 2003).

Structure and function of ARs

Androgenic regulation of gene expression is mediated via the binding of androgens to their cognate AR (NR3C4). The gene encoding human AR is located on the X chromosome (Lubahn *et al.* 1988). Cloning has identified eight exons with conserved domains in common with other steroid hormone receptors (Kuiper *et al.* 1989). The full-length human AR protein (110 kDa) has an extensive amino-terminal domain (NTD), a DNA-binding domain, a hinge region and a ligand-binding domain. Intra- and inter-molecular interactions between domains are important for the receptor's activation capacity (reviewed in Claessens *et al.* (2008)).

One of the most studied polymorphisms of the AR NTD is the CAG-repeat sequence encoding poly-glutamine repeats (Spada *et al.* 1991). The length of the CAG-repeat region correlates inversely with AR transactivation capability (Chamberlain *et al.* 1994). Notably, an association has been reported between the number of CAG and GGN repeats in the NTD and the progression state of EC, with a prevalence of short repeats in more benign tumours (McGrath *et al.* 2006, Rodríguez *et al.* 2006). However, it should be noted that a later study reported a weak association between the CAG repeats and EC risk (Yang *et al.* 2009). Epigenetic regulation of the AR gene has also been implicated in endometrial malignancy, with hypermethylation of a CpG region spanning the transcription start site being associated with AR gene inactivation in EC patients (Sasaki *et al.* 2000). Loss of AR expression in stages III and IV of the disease also correlates with the methylation status of this CpG region, with cancerous and normal tissues from the same patient having a significant difference in both methylation status and the presence/absence of AR expression (Sasaki *et al.* 2000).

AR is the target of several post-translational modifications including acetylation, phosphorylation, methylation, ubiquitination and sumoylation (reviewed in Coffey & Robson (2012)). AR sumoylation has been demonstrated to enrich AR in the nuclear matrix under conditions of cellular stress, causing attenuation of the transcriptional activity of AR, contributing to defective androgen signalling in cancer (Poukka *et al.* 2000, Rytinki *et al.* 2012). Phosphorylation of serine residues in the NTD of AR can result from ligand activation as well as growth factor and other signalling cascades, which in turn can influence the secondary structure of the domain (reviewed in Kumar & McEwan (2012)). It is notable that phosphorylated AR proteins have been localised in both ovarian tissues (McEwan *et al.* 2010) and recently investigated in the context of breast cancer (Ren *et al.* 2013).

Expression of ARs in the non-malignant endometrium and ovary

Expression of ARs has been documented throughout the female reproductive system including the ovary (Saunders *et al.* 2000), fallopian tube (Horne *et al.* 2009) and endometrium (Marshall *et al.* 2011) consistent with a role for local or peripheral androgens in modulating the function of these tissues.

Endometrium

It has been reported that ARs are expressed in the endometrium throughout the menstrual cycle (Mertens *et al.* 2001, Apparao *et al.* 2002, Marshall *et al.* 2011). In our own studies examining immunoexpression of AR in full-thickness sections of human endometrium (see supplementary Fig. 1 in Marshall *et al.* (2011)), we have noted the highest levels of immunoexpression in the stromal cells within the upper, functional, layer during the oestrogen-dominated proliferative phase with immunopositive stromal cells in the basal layer throughout the cycle regardless of stage. Although some authors have claimed that AR is detected in glandular epithelium throughout the menstrual cycle (Horie *et al.* 1992), we and others have reported that immunoexpression is up-regulated in the glandular epithelium in the mid/late secretory phase at a time when progesterone concentrations are falling due to the demise of the corpus luteum (Marshall *et al.* 2011).

The generation of a transgenic *Ar* reporter mouse, which expressed a luciferase reporter gene under the control of activated endogenous AR, has revealed high AR activity in both the uterus and the ovary,

demonstrating the direct action of androgens in these tissues (Dart *et al.* 2013). Studies on AR knockout (ARKO) female mice have shown that they have normal reproductive tracts under macroscopic examination but are sub-fertile (Hu *et al.* 2004). A study utilising reciprocal paired ovarian transplantation between $Ar^{+/+}$ and $Ar^{-/-}$ mice revealed that host $Ar^{-/-}$ female mice with $Ar^{+/+}$ ovarian transplants had defective uterine growth consistent with an intrauterine role for androgens in this species (Walters *et al.* 2009).

Ovary

ARs are expressed in somatic cells within the human ovary in both foetal and adult life (Saunders *et al.* 2000, Fowler *et al.* 2011). It is notable that ARs are present in cells of the surface epithelium, as malignant transformation of this cell type has been implicated in development of epithelial ovarian cancers following formation of inclusion cysts (Rabban & Bell 2005) although which subtypes this is associated with is currently being re-evaluated (Berns & Bowtell 2012). In all species examined to date, intense AR immunoreexpression has been documented in adult granulosa cells (Saunders *et al.* 2000). In primate ovaries, expression of AR was most abundant in immature follicles (Hillier *et al.* 1997).

ARKO mice generated by targeted deletion of exon 2 have longer oestrous cycles and fewer corpora lutea, and when superovulated they produced fewer oocytes compared with WT. Intense granulosa cell apoptosis was detected in pre-ovulatory follicles (Hu *et al.* 2004). Similarly, ARKO mice with an inframe deletion of exon 3 (DNA-binding zinc finger 2) are reported to be subfertile with reduced ovulation associated with morphologically unhealthy antral follicles (Walters *et al.* 2007). Female mice with targeted deletion of *Ar* in granulosa are also subfertile confirming the importance of AR expression in granulosa cells for normal ovarian function (Walters *et al.* 2012).

Thus, androgen action is an important feature of normal reproductive function and AR-dependent signalling influences uterine growth and ovarian function.

Androgen action in EC and ovarian cancer

AR expression in EC

There have been limited studies describing the expression of AR in EC. Horie *et al.* (1992) described positive immunostaining for AR in glandular cells and in the solid mass in a small sample set of grade II

adenocarcinomas. Ito *et al.* (2002) compared the expression of AR and 5 α -reductase types 1 and 2 in 44 cases of endometrioid adenocarcinomas. Positive immunostaining for AR was detected in stromal cells and in 88.6% of carcinoma cells, while 5 α -reductase types 1 and 2 were detected in ~80% of carcinoma cells. Analysis of endometrioid carcinoma tissue homogenates confirmed mRNA expression of AR, 5 α -reductase types 1 and 2 in the majority of cases (Ito *et al.* 2002). By contrast, Sasaki *et al.* (2000) detected AR-positive cells in only 19 of 89 EC tissues, although the cancer samples investigated were not restricted to a single histological type. A recent analysis of uterine sarcomas failed to identify AR expression in uterine carcinosarcoma, leiomyosarcoma or endometrial stromal sarcoma (Koivisto-Korander *et al.* 2011).

AR protein levels are reported to decline as EC progresses from well- to poorly differentiated tumours (Kato & Seto 1985). In our own laboratory, we consistently detect AR in the nuclei of epithelial and stromal cells in stage I tumours graded as well or moderately well differentiated (Fig. 1A, B, C and D). Notably, AR staining was not always evenly distributed within all epithelial cells (Fig. 1C) and was sporadic in poorly differentiated tumours that lacked a defined epithelial compartment (Fig. 1F). Nuclear AR was detected in stromal cells in all stages but vascular and immune cells were immunonegative (asterisks in Fig. 1D and E).

Results reported for some endometrial cell lines may be unreliable due to cross-contamination with other cancer cells (Korch *et al.* 2012). The most widely used cell line, the Ishikawa cell line (available from ECAC-C/ATCC), was derived from a moderately differentiated stage II endometrial adenocarcinoma tumour (Nishida *et al.* 1996). We, and others (Lovely *et al.* 2000), have consistently found that this cell expresses AR, ER α (ESR1) and ER β (ESR2). Notably, it has been reported that AR protein expression in this transformed epithelial cell can be induced by oestradiol (E $_2$) or DHT but is down-regulated by medroxyprogesterone acetate (MPA) or the anti-androgen hydroxyflutamide (Apparao *et al.* 2002).

AR expression in ovarian cancer

There have been a number of studies showing over-expression of AR in ovarian cancer with papers also reporting the impact of androgens on gene expression and survival of ovarian cancer cell lines (reviewed in Modugno *et al.* (2012)). In a recent study, positive AR immunostaining was reported in two sets of ovarian cancer tissues. The histology of 46 serous epithelial cancers

Figure 1

Androgen receptor expression in stage I endometrial adenocarcinomas. Endometrial cancer cells from a dataset described previously in [Collins et al. \(2009\)](#) were immunostained using rabbit anti-human AR (Abcam, Cambridge, UK) in 1:100 dilution (see [Marshall et al. \(2011\)](#), with detection using DAB as described in [Collins et al. \(2009\)](#)). Intense nuclear expression of AR was detected in epithelial cells in cancers graded as well (A and B), moderately (C and D) and poorly (E) differentiated. Epithelial AR staining was heterogeneous with some epithelial cells immunonegative for AR

(arrowheads). The number of immunopositive cells was highly variable and immunopositive cells were not evenly distributed throughout the section (see C). Immunopositive fibroblasts were also noted within the stroma (st), but putative immune cells within blood vessels (v) were immunonegative (**). In poorly differentiated tumours (F) that lacked a defined epithelial compartment, AR immunostaining was sporadic (arrows). Magnifications: (A and E), $\times 20$ and (B, C, D and F), $\times 40$.

(patients aged 27–86) as well as 23 samples with matched metastases was assessed and the majority demonstrated moderate to strong nuclear immunostaining for AR ([Butler et al. 2013](#)). This study also examined expression of an AR nuclear chaperone called SGTA and reported a correlation between AR:SGTA ratio and disease subtype ([Butler et al. 2013](#)). In epithelial ovarian cancers, it has been reported that women with shorter CAG repeats in the NTD of their

AR had decreased overall survival ([Li et al. 2003](#)). Other reports examining expression of the AR co-activator AIB1 (NCOA3) have reported that isoforms of the protein with shorter CAG repeats are associated with poor prognosis ([Li et al. 2005](#)). Other AR co-activators implicated in ovarian cancers such as p44/Mep50/WDR77 may also act as co-activators for oestrogen receptors, making it difficult to know whether over-expression in ovarian cancers and

associated increases in cell proliferation and invasion are AR mediated (Ligr *et al.* 2011).

Androgen metabolism and the impact on androgen bioavailability

The greatest concentrations of circulating androgens are precursors/prohormones that can be activated locally by the action of 5α -reductase (testosterone to DHT) and 17β -hydroxysteroid dehydrogenases (17β HSD) enzymes (A4 to testosterone). The expression and activity of steroid-metabolising enzymes determine the local bioavailability of androgens and thus affect AR-dependent signalling in normal and malignant tissues. The transcriptional activity of ARs is altered by endogenous androgens such as testosterone and DHT. DHT is the most potent endogenous AR agonist, while A4 and DHEA have extremely low binding affinity for AR (Avances *et al.* 2001). Studies investigating 5α -reductase protein expression in the endometrium have reported expression in epithelial cells alone (Ito *et al.* 2002) or both stromal and epithelial cells (Carneiro *et al.* 2008). In studies using human endometrial explants (Rose *et al.* 1978), 5α -reductase has also been shown to reduce testosterone to DHT consistent with local androgen activation in intact tissue. The endometrium is also reported to convert the adrenal androgen DHEAS to DHEA and A4 (Hausknecht *et al.* 1982). Interestingly, a recent study has shown that A4 up-regulates aromatase mRNA expression in human endometrial stromal cells and explants (Bukulmez *et al.* 2008) underlining the importance of local bioavailability and metabolism of androgens in modulating the steroid microenvironment in the endometrium.

Tumour-associated steroid metabolism in EC and ovarian cancer

Dysregulation of enzymes that mediate steroid metabolism is an important feature of the pathophysiology of reproductive malignancies. Rizner *et al.* (2006) showed that dysregulation of AKR1C3 (also known as 17β HSD5), which converts A4 to testosterone, is associated with EC with increased mRNA expression in malignant endometrium compared with normal endometrium. A recent review from the same group concluded that due to the limitations of the available studies on AKR1C3 protein expression, the importance of AKR1C3 in EC pathophysiology requires further investigation (Rizner 2013). Sinreih *et al.* investigated the expression of progesterone synthesis and metabolism genes in 47 stage I EC tumours

and found decreased expression of *CYP11A1* and *STAR* in EC tumours, which may indicate that local steroid metabolism/conversion rather than circulating hormone concentrations are important in determining intra-tumoural hormone bioavailability. Interestingly, a 3.7-fold decrease in *SRD5A2* expression and a threefold increase in *HSD17B2* expression in tumour compared with adjacent control endometrial tissue were also reported consistent with a potential for altered intra-tumoural androgen metabolism (Sinreih *et al.* 2013). This altered expression would be consistent with decreased activation of androgens as a result of less conversion of testosterone to DHT (5α -reductase activity) and increased conversion of testosterone to A4 (17β HSD2 activity) within EC. Interestingly, A4 is the preferential substrate for aromatase, which might suggest that altered metabolism of androgens favours an oestrogenic pathway. Notably, concentrations of E_2 measured in EC tumour tissues are higher than those of disease-free endometrium (Berstein *et al.* 2003).

The activity of steroid sulphotase (STS) enzymes may also increase bioavailable androgens through de-sulphation of DHEAS to DHEA. Expression and activity of STS has been reported in endometrial carcinoma cell lines (Smuc *et al.* 2006, Fournier & Poirier 2009). STS activity is reported to be higher in EC tissue compared with normal endometrium (Yamamoto *et al.* 1993), and more recent studies have reported that STS mRNA levels are increased in tumoural endometrial tissues compared with peri-tumoural endometrial tissues (Lepine *et al.* 2010). In addition, Abulafia *et al.* (2009) reported significantly increased DHEAS sulphotase activity in stage I endometrioid carcinoma compared with normal endometrium consistent with a role for increased activation of androgen precursors in EC.

Variants in genes involved in the synthesis and action of sex steroid hormones are likely to be important in the aetiology of EC. The association between polymorphisms in genes encoding sex steroid enzymes and steroid receptors and EC risk has been examined in a number of studies with the focus directed towards genes involved in the oestrogen biosynthesis pathway. An association between single nucleotide polymorphisms (SNPs) in *CYP19A1* with oestrogen concentrations (both oestrones (E_1) and E_2) and oestrogen:testosterone ratios has been described in post-menopausal women (Dunning *et al.* 2004). Olson *et al.* (2007) reviewed the association between variants in steroid biosynthetic genes, circulating hormones and EC risk by summarising available data for seven genes in the oestrogen biosynthesis pathway. Variants of *CYP11A1*, *CYP17A1* and *HSD17B1* were not associated

with altered levels of progesterone, androgens or oestrogens; *CYP17A1* variants were associated with a decreased risk of EC and *CYP19A1* variants with an increased risk of EC (Olson *et al.* 2007). A recent case-control study of polymorphisms in 391 EC cases and 712 individually matched controls found that *CYP19A1* variants are associated with an increased risk of EC (Lundin *et al.* 2012). Interestingly, in an extensive analysis of common genetic variation of 36 sex hormone-related genes by Yang *et al.* (2010), genetic variation in *AR* was significantly associated ($P=0.006$) with an increased risk of EC.

Alterations in the expression/activity of ovarian steroidogenic enzymes may also contribute to ovarian cancer risk. GCTs are rare (2–5% of all ovarian cancers) but characterised by their ability to synthesise and secrete steroids including oestrogens and androgens with some post-menopausal patients presenting with symptoms including hirsutism and virilisation (Schumer & Cannistra 2003). In mice, over-expression of human *HSD17B1* resulted in development of an androgen-dependent ovarian benign serous cystadenoma phenotype in adulthood (Saloniemi *et al.* 2007). Beesley *et al.* (2007) assessed putative SNPs in genes involved in steroid hormone synthesis and found an increased risk of epithelial ovarian cancer associated with a SNP in the 5 α -reductase gene (*SRD5A2* V89L). However, a subsequent analysis by the ovarian cancer consortium in a large pooled sample of 4624 invasive epithelial cancer cases and 8113 controls found no association between *SRD5A1* and ovarian cancer risk (Ramus *et al.* 2008).

Taken together, these data suggest that dysregulation of androgen biosynthesis is a feature of the pathophysiology of EC. The association between ovarian cancer and altered steroid metabolism is less clear with interpretation limited by heterogeneity of the disease.

Androgens and ARs as a therapeutic target in EC and ovarian cancer

Endometrial cancer

Despite the potential associated risks of elevated androgens (described above), androgens receptors may be a potential therapeutic target in EC. For example, testosterone treatment of post-menopausal women does not appear to induce endometrial stimulation and decreases the proliferative effect of E_2 (Zang *et al.* 2007). Studies on cancer cell lines have shown that AR-dependent signalling inhibits proliferation of cells derived from breast and endometrial tumours (Hackenberg & Schulz 1996).

In a mouse model of endometrial carcinogenesis, the synthetic androgen Danazol significantly decreased expression of the proliferation marker PCNA and the incidence of endometrial hyperplasia (Niwa *et al.* 2000).

Treatment of primary endometrial stromal cells with DHT alters expression of genes involved in proliferation, cell survival and migration, all of which are likely to be dysregulated in tumours (Marshall *et al.* 2011). Therefore, targeting AR-regulated transcription could be a productive therapy for preventing disease progression. Interestingly, cyclin D1 (*CCND1*), a protein which is important in regulating the cell cycle is reported to be AR regulated (Lanzino *et al.* 2010). Expression of cyclin D1 has been linked to breast cancer growth and progression, and studies on breast cancer cells have revealed that *CCND1* promoter activity can be inhibited by DHT-activated AR (Lanzino *et al.* 2010). *CCND1* expression is significantly associated with EC grade (Moreno-Bueno *et al.* 2003) with a recent study suggested that cyclin D1 may be a prognostic marker for endometrial diseases with over-expression of cyclin D1 in EC and atypical complex hyperplasia (Liang *et al.* 2013). A recent study investigating changes in Ishikawa cells in response to tamoxifen using gene expression profiling found that this synthetic SERM significantly up-regulated *CCND1* (Tamm-Rosenstein *et al.* 2013). As tamoxifen exposure is associated with an increased risk of EC, androgen-based therapies that promote down-regulation of *CCND1* expression may decrease risk of disease development.

Prostate-specific antigen (*PSA/KLK3*) is an androgen-regulated gene that has been extensively investigated in the context of prostate cancer (Montgomery *et al.* 1992). Mhaweche-Fauceglia *et al.* (2008) assessed PSA expression in 49 endometrial adenocarcinoma tissue specimens. The study reported weak expression of PSA in the glands of normal endometrium and that 67.7% of EC tissues had no/weak staining for PSA. High PSA mRNA levels were associated with stage I disease but not with tumour grade or subtype. Interestingly, multivariate survival analysis associated loss of PSA expression with worse disease-free survival (Mhaweche-Fauceglia *et al.* 2008). Circulating levels of PSA are increased in women with PCOS and are directly correlated with hyperandrogenism (Mardanian & Heidari 2011). As AR expression decreases with progression of EC, loss of AR-regulated PSA may be important in EC disease progression.

Mutations in the oncogene *KRAS* are associated with EC and can lead to stimulation of the ERK1/2 signalling pathway in the absence of stimuli and activating *KRAS* mutations have been identified in precursor lesions for

ECs (Dobrzycka *et al.* 2009). A recent integrated analysis of *KRAS* CNA and mutations found that increased *KRAS* copy number and mRNA expression but not *KRAS* mutations were associated with EC disease progression and poor disease-specific survival (Birkeland *et al.* 2012). The authors report that mRNA expression levels and *KRAS* amplification were increased significantly in metastatic compared with primary lesions consistent with involvement of *KRAS* alterations in disease progression (Birkeland *et al.* 2012). In a recent study, *KRAS* protein expression was reported to be decreased by AR signalling in ER–, PR– and AR+ breast cancer cells (Lyu *et al.* 2014), which may indicate a further target for androgen therapy in EC.

The tumour suppressor gene phosphatase and tensin homologue deleted on chromosome 10 (encoded by *PTEN*) regulates cell growth, apoptosis and proliferation (Zhao *et al.* 2004). Mutations in *PTEN* are common in EC and this leads to inactivation and loss of *PTEN* expression, which is an early diagnostic marker for endometrial pre-cancers (Mutter *et al.* 2000a). In the normal endometrium, *PTEN* appears to be under hormonal control (Mutter *et al.* 2000b). Guzeloglu-Kayisli *et al.* (2003) reported that E₂ promoted *PTEN* phosphorylation and that progesterone increased *PTEN* protein expression in isolated endometrial, stromal and epithelial cells. The impact of androgens on *PTEN* expression in the endometrium is unknown and likely to be cell context dependent as AR-dependent signalling inhibits *PTEN* expression in prostate cancer cells whereas stimulation of AR signalling up-regulates *PTEN* expression in breast cancer cells (Wang *et al.* 2011).

AR-dependent regulation of gene expression may play a role in progression of EC; however, as responses are likely to be cell context dependent, further investigation is required to fully understand which target genes are affected.

Ovarian cancer

In contrast to the potential anti-proliferative role of androgens in breast and EC, androgens may promote growth of some ovarian cancers and anti-androgen therapy has been proposed. *In vitro*, androgens promote proliferation of ovarian surface epithelial (OSE) cells and human ovarian cancer cells (Syed *et al.* 2001). Some studies on androgen action in ovarian carcinomas may need to be treated with caution as some of the established cell lines may not be of ovarian cancer origin (Korch *et al.* 2012). In a recent review, Modugno *et al.* (2012) summarised the evidence that therapies targeting ARs for treatment of women with ovarian cancer might be a promising

strategy. They highlighted the small scale of most trials; they had used anti-androgens for treatment of women with recurrent cancer and variable results were obtained. When Elattar *et al.* (2012) tested the impact of DHT on cell division in primary ovarian epithelial cancer cells isolated from the ascites of 11 patients with advanced primary ovarian cancers, they recorded variable concentrations of AR mRNA and an increase in S-phase cells, which was abrogated by treatment with anti-androgen. They also noted a decrease in AR positive staining of tumours after chemotherapy and concluded that, although there might be a subgroup of patients that could benefit from anti-androgen therapy, this might be best administered early in the treatment regime.

In granulosa cell cultures, Stocco *et al.* have reported that testosterone, but not DHT, alters expression of aryl hydrocarbon receptor (AHR) and liver receptor homologue 1 (LRH1 (NR5A2)) with an AHR-dependent interaction between AHR and AR leading to increased expression of the *LRH1* gene (Wu *et al.* 2013). As LRH1 is an orphan receptor that has been implicated in promoting invasion and migration of breast cancer cells independent of oestrogen action (Chand *et al.* 2010), any increase in expression as a result of androgen action might promote invasiveness of granulosa cells and it is notable that LRH1 level is elevated 30-fold in GCTs (Chand *et al.* 2013). Consistent with their cell of origin, analysis of GCTs and derived cell lines (COV434 and KGN) confirmed expression of several steroid hormone receptors including AR and ER β (Alexiadis *et al.* 2011). Notably, treatment of a small group of patients with diagnosis of a GCT with aromatase inhibitors has reported positive results (Alhilli *et al.* 2012).

Microarray experiments using DHT-stimulated OVCAR3 cells (established from ascites of a woman with an ovarian adenocarcinoma) reported that 121 genes were up-regulated (Sheach *et al.* 2009) including Ras-related protein, RAB25 (*RAB25*), a small GTPase implicated in vesicle trafficking and cancer metastasis previously shown to be over-expressed in aggressive cancers (Cheng *et al.* 2004). A recent report highlighted a role for promoter methylation in the regulation of expression of the AR co-activator melanoma-associated antigen 11 (*MAGEA11*) in ovarian and other cancers, a finding that is important as expression is correlated with poor prognosis (James *et al.* 2013). In a recent study of ~3000 women with a mean age of ~57 years, invasive epithelial ovarian cancer tissues were examined to determine whether there was a link between expression of progesterone receptor or oestrogen receptor α and subtype-specific survival (Sieh *et al.* 2013a);

although the study did not measure AR, it reported that expression of PR (PGR) was associated with improved survival with high-grade serous carcinoma. A number of clinical trials have used endocrine therapies to reduce oestrogen production (e.g. aromatase inhibitors) or receptor activation with mixed results (reviewed in [Modugno *et al.* \(2012\)](#)). Treatment of women with aromatase inhibitors has the potential to raise local and peripheral androgens by blocking their conversion to oestrogens, hence further studies are needed to see if changes in bioavailable androgens as a result of this treatment regime contribute positively or negatively to disease outcome.

Insights into the role of androgens in breast and prostate cancers

Studies investigating the role of AR in breast cancer suggest that focusing on AR-dependent transcriptional activity alone will not be sufficient to fully understand how endogenous androgenic ligands, or the use of AR modulators, might alter cancer development/progression in EC or ovarian cancer. For example, studies on the breast have considered two aspects: anti-androgenic effects of the progestin MPA ([Ochnik *et al.* 2014](#)) and crosstalk between AR and ER α signalling due to overlapping binding sites within DNA ([Need *et al.* 2012](#), [Ochnik *et al.* 2014](#)). Studies on breast tissue from post-menopausal women showed that DHT had an anti-proliferative effect that was opposed by MPA (a component of some HRT formulations) due to destabilisation of AR and increased proliferation ([Ochnik *et al.* 2014](#)). Following reports that DHT could induce proliferation of ER α -positive and ER α -negative breast cancer cell lines via distinct mechanisms ([Lin *et al.* 2009a](#)), [Need *et al.* \(2012\)](#) used the ChIP-Seq technology to demonstrate reciprocal interference between DHT- and E $_2$ -induced gene expression profiles and altered expression of genes such as progesterone receptor, *FKBP5* and *CXCL12*, all of which are considered as classically regulated by oestrogens. Importantly, we have previously reported significantly decreased concentrations of ER α mRNA in poorly differentiated tumours compared with both well- or moderately differentiated grade I ECs and demonstrated that ER α protein expression was low/absent in poorly differentiated tumours ([Collins *et al.* 2009](#)). We postulate that changes in the relative expression ratios of AR and ER α are likely to impact on the ability of androgens to activate/repress expression of genes implicated in key processes such as cell migration, proliferation and apoptosis.

Studies on prostate cancer cells suggest that the transforming growth factor β (TGF β) signalling molecule

Smad3 can transactivate AR and it has been reported that DHT can suppress the ability of TGF β to promote apoptosis. Additional data examining the AR-associated protein HIC5 (TGFB1I1) in this interaction highlight a complex interplay between these signalling pathways and cancer progression ([Wang *et al.* 2005](#), [Song *et al.* 2010](#)). Androgen signalling may also influence epithelial to mesenchymal transition (EMT), a critical event in cancer metastasis. Recent studies on prostate cancer epithelial cells indicate that androgens induce EMT, migration and invasion of prostatic epithelial cells ([Zhu & Kyprianou 2010](#)).

In addition to transcriptional crosstalk between ER α and AR, testosterone has been shown to induce ERK and Akt phosphorylation by activating the ER α splice variant ER α 36 in the ER α - and AR-EC cell line Hec1A ([Lin *et al.* 2009b](#)). Thus, androgens may stimulate activation of signalling pathways via ER splice variants in the absence of AR that may be important in the context of decreased AR expression with EC disease progression.

Future perspectives

To date, information on the potential impact(s) of androgens on aetiology and prognosis in EC and ovarian cancer has largely focused on the potential for intra-tumour biosynthesis of ligand and on the use of transformed cell lines for examination of androgen-dependent gene expression (see above).

Androgen-dependent signalling may affect the expression of oncogenes, tumour suppressor genes, cell cycle regulators and metastasis-associated genes that may impact on disease progression. Information from studies on other cancers suggest an extensive capacity for cross-talk between steroid receptors. Therefore, detailed sequencing analysis will be required to understand the distinct gene sets regulated by oestrogens, androgens and progestogens in a given tissue, and measuring intra-tissue levels of sex steroid hormones will be required to estimate the possible converging signalling inputs that may influence malignant development within tissues. The effect of AR-dependent signalling will be determined by AR expression, cell context expression of co-regulatory proteins, expression and activity of other sex steroid receptors that may compete for DNA-binding sites on the promoters of target genes as well as the local bioavailability of ligand for the receptor. In order to understand the relative contribution of androgen action to risk of malignancy, the balance in expression and activity of steroid enzymes within the tissue, which determines AR ligand bioavailability, needs further

investigation. testosterone and A4 can be interconverted by the action of 17 β HSD isozymes and testosterone can also be reduced by 5 α -reductase to more potent androgen DHT. While DHT is non-aromatisable, both testosterone and A4 can be aromatised to active oestrogens. Thus, a better understanding of the relative contribution of 5 α -reductase and aromatase enzyme activity is required in order to ascertain whether increased androgen concentrations have a direct effect on the tissue or whether they are acting as pro-hormones to increase high-risk oestrogen exposure.

Studies on the role of androgens in the context of ovarian cancer would be particularly timely as the new findings, suggesting that many forms of epithelial ovarian cancer previously thought to originate from OSE cells may actually derive from extra-ovarian sources (reviewed in Kurman & Shih Ie (2011)). For example, cells within the fallopian tube epithelium have been proposed as the cell type(s) contributing to low- and high-grade serous carcinomas (Piek et al. 2001). A mouse model using a conditional double KO of *Dicer* (*Dicer1*) and *Pten* demonstrated that high-grade serous cancers develop in the fallopian tube and metastasise to the ovary (Kim et al. 2012). Although there is still evidence to support OSE cells as the originating cell type for some ovarian cancers (Auersperg 2013), the fact that both OSE cells and cells within the fallopian tube epithelium express AR (Edmondson et al. 2002, Horne et al. 2009, Mendez et al. 2013) highlights the potential for androgens to modulate disease development and progression. Stem-like epithelial cells from the distal end of the fallopian tube capable of clonal growth and self-renewal have also been identified which may play a role in the initiation of serous tumours, although the impact of androgens on this cell population has not been investigated (Paik et al. 2012). The associated increased risk of ovarian cancer in women with endometriosis (Pearce et al. 2012) may suggest that AR-positive cells in endometrial tissue might also contribute to development of ovarian cancer as AR protein and mRNA have been reported in both eutopic endometrium and peritoneal lesions in women with peritoneal endometriosis (Carneiro et al. 2008).

Improving our understanding of the tissue-specific effects of AR signalling may lead to the application of novel therapeutic strategies such as combination therapies with aromatase inhibitors or SERMs (see data above on crosstalk with ER α). In addition, a novel 45 kDa AR isoform (AR45) that was discovered in 2005 was found to be expressed in the uterus and shown to be a negative regulator of AR by interacting with the AR NTD (Ahrens-Fath et al. 2004).

Elucidating the expression of this variant in normal and malignant reproductive tissues may lead to the identification of a potential therapeutic target.

Using computational pharmacophore modelling and virtual screening, second-generation anti-androgens have been developed which could be of therapeutic benefit in treating AR-dependent ovarian cancers (Voet et al. 2013). Furthermore, the generation of selective AR modulators (SARMs) has revolutionised the potential manipulation of androgenic stimulation in a therapeutic context (Gao & Dalton 2007). The important role played by androgens in regulation of muscle mass and aetiology of prostate cancer has led to development of compounds that are classified as SARMs and are designed to maintain well-being in men without causing prostatic hyperplasia or malignancy. A recent review highlighted positive impacts of SARMs on cancer-associated muscle wasting (Dalton et al. 2013). Although limited evidence has been presented to date, press reports on one SARM (Enobosarm, Gtx, Inc., Memphis, TN, USA) highlight preliminary (unpublished) data of a trial testing use as a therapy for women with metastatic breast cancer (<http://phx.corporate-ir.net/phoenix.zhtml?c=148196&p=irol-newsArticle&ID=1884210>). The use of SARMs could be of great potential therapeutic benefit in the treatment of gynaecological malignancy. A SARM with the anti-proliferative effects of androgens in the endometrium but without the proliferative effect of androgens on OSE cells could have potential therapeutic merit in treatment of both EC and ovarian cancer.

Summary and conclusions

The impact of androgen action in EC and ovarian cancer is poorly understood. Epidemiological evidence suggests that increased risk of EC is associated with increased exposure to androgen action through elevated circulating concentrations of testosterone, conditions that may cause hyperandrogenism such as PCOS, as well as genetic variation in the AR. Cell and animal studies suggest that androgens may be a beneficial therapeutic target in EC with the potential to inhibit proliferation and promote changes in expression of oncogenes, cell cycle regulators, tumour suppressor genes and metastasis-associated genes.

The evidence for a role for androgen action in ovarian cancer is less clear and is limited by the heterogeneous nature of the disease. Obesity is associated with an increased risk of ovarian cancer and some studies suggest that obesity may promote a hyperandrogenic state. In addition, limited studies suggest that exposure to the

synthetic androgen Danazol increases risk of ovarian cancer. Analyses of SNPs in genes associated with the androgen biosynthetic pathway suggest that variants of *SRD5A1* may be associated with epithelial ovarian cancer. Androgens may be a key therapeutic target in ovarian cancer, as anti-androgens have been shown to inhibit proliferation of ovarian cancer cells in some studies.

Analysis of intra-tumoural steroid metabolism suggests that androgen metabolism is altered and may promote formation of oestrogens, but it is not currently clear whether androgens have a direct impact on the tumour or whether they act solely as precursors to oestrogens. A better understanding of the local, intracrine activity of enzymes in affecting bioavailability of sex steroids in order to most effectively utilise combination therapies or new therapeutic agents such as SARMS is required. Further investigation will be required to understand the cell context-dependent impact of AR signalling and how AR-dependent signalling may crosstalk with other steroid receptors in the development and progression of disease.

Declaration of interest

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

Funding

This work was supported by the Medical Research Council (grant number G1100356/1).

Author contribution statement

All authors contributed to the planning and writing of this review. P T K Saunders prepared the figure.

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Received in final form 17 February 2014

Accepted 24 February 2014

Made available online as an Accepted Preprint

28 February 2014