Complexities of androgen receptor signalling in breast cancer

Keely M McNamara, Nicole L Moore, Theresa E Hickey, Hironobu Sasano and Wayne D Tilley

Department of Pathology, Tohoku University School of Medicine, Miyagi, Sendai, Japan
1Dame Roma Mitchell Cancer Research Laboratories, Discipline of Medicine, The University of Adelaide
and Hanson Institute, DX 650801, Adelaide, South Australia 5005, Australia
*(K M McNamara and N L Moore contributed equally to this work)

Abstract

While the clinical benefit of androgen-based therapeutics in breast cancer has been known since the 1940s, we have only recently begun to fully understand the mechanisms of androgen action in breast cancer. Androgen signalling pathways can have either beneficial or deleterious effects in breast cancer depending on the breast cancer subtype and intracellular context. This review discusses our current knowledge of androgen signalling in breast cancer, including the relationship between serum androgens and breast cancer risk, the prognostic significance of androgen receptor (AR) expression in different breast cancer subtypes and the downstream molecular pathways mediating androgen action in breast cancer cells. Intracrine androgen metabolism has also been discussed and proposed as a potential mechanism that may explain some of the reported differences regarding dichotomous androgen actions in breast cancers. A better understanding of AR signalling in this disease is critical given the current resurgence in interest in utilising contemporary AR-directed therapies for breast cancer and the need for biomarkers that will accurately predict clinical response.

Key Words
- androgen
- breast cancer
- intracrinology
- risk
- outcome
- androgen regulated pathways

Introduction

The importance of androgenic hormones in breast cancer has been recognised for much of the last century. Despite little understanding of their cellular actions in breast tissue at the time, androgenic compounds were used as breast cancer therapies between the 1940s and the 1980s. Although this therapeutic strategy displayed sound clinical efficacy (Tormey et al. 1983, Ingle et al. 1991), the use of androgen-based hormonal therapies fell from favour due to their masculinising side effects in some women and the concurrent development of targeted anti-oestrogenic therapies. With recent insights into the molecular heterogeneity of breast cancers, intracrine steroid metabolism and mechanisms of anti-oestrogen therapy resistance, an appreciation of the role of androgen signalling pathways in breast cancer is experiencing a revival. For example, contemporary pre-clinical studies have led to clinical trials utilising androgen receptor (AR) antagonists as breast cancer therapies for women with metastatic disease. In this review, current knowledge on the role of androgen signalling in breast tumorigenesis will be discussed, indicating the complexities of androgen action in different breast cancer subtypes and the potential to exploit this pathway for successful new therapeutic interventions.

Androgens and breast cancer risk

Although androgens are commonly considered as male hormones, they are also detected at physiologically
relevant levels in the circulation of women (Burger 2002, McNamara et al. 2010) and play important biological roles in females. There are several species of androgenic hormones in the circulation including, in the order of high to low concentration, dehydroepiandrosterone-sulphate (DHEAS), DHEA, androstenedione (A₄), testosterone and 5α-dihydrotestosterone (DHT). The potent androgen testosterone and, to a lesser extent, the precursor androgens DHEA and A₄ are produced by the ovaries while DHEA, DHEAS, A₄ and testosterone are secreted by the adrenal glands. In addition to these endocrine sources, testosterone, DHT and their metabolites are also synthesised in peripheral tissues such as the breast, bone and brain (Labrie et al. 2003). Testosterone levels fluctuate during the menstrual cycle and gradually decline, although not completely, following menopause (Labrie et al. 2003, Rothman et al. 2011). By contrast, adrenal androgen production continues following menopause (Labrie et al. 2003). Both androgens and oestrogens bind to steroid hormone-binding globulin (SHBG) in the circulation, which controls the bioavailability of hormones to the breast and other tissues. Numerous studies have examined the potential role of circulating androgens as risk factors for breast cancer, as discussed in detail below. In general, elevated serum testosterone level has been associated with an increased breast cancer risk in postmenopausal women in some studies; however, results between studies are often contradictory and the relationship remains unclear. This is largely due to the inherent limitations in the techniques used to measure testosterone levels and the difficulty in interpreting these studies in relation to intracrine steroid metabolism (see sections ‘Limitations in the measurement and interpretation of serum testosterone levels and breast cancer risk’ and ‘Intracrine androgen metabolism in the breast’).

Premenopausal women

Nine large-scale prospective cohort studies have examined endogenous serum testosterone levels and breast cancer risk in premenopausal women. Four of these studies showed significantly higher levels of serum testosterone in breast cancer patients compared with controls, while three of these four studies also found a significantly increased breast cancer risk associated with increased testosterone levels (Table 1 and references therein). The remaining studies found no significant association between serum testosterone and breast cancer risk (Table 1). Furthermore, five studies also measured bioavailable testosterone (the component of steroid calculated to be unbound to SHBG), as opposed to total testosterone that also includes the component bound to SHBG. Two of the five studies reported a significant positive association between bioavailable testosterone and breast cancer risk (Supplementary Table S1, see section on supplementary data given at the end of this article). In addition to testosterone, some of these studies also measured DHEAS, A₄, 17β-oestradiol (E₂), oestrone (E₁) or SHBG, but an association with breast cancer risk was rarely observed for these variables in premenopausal women (Supplementary Table S1).

Postmenopausal women

The association between endogenous circulating testosterone and breast cancer risk in postmenopausal women has been reported in 11 large-scale prospective cohort studies. The majority of these studies reported that higher levels of circulating testosterone were significantly associated with an increased breast cancer risk (Table 1 and references therein). Similarly, higher levels of other hormones, including free testosterone, DHEAS, A₄, E₂ or E₁, were associated with an increased breast cancer risk in several of these studies, while reduced levels of SHBG were sometimes associated with a decreased risk of breast cancer (Supplementary Table S1 and references therein). An association between increased testosterone and breast cancer recurrence has also been reported (Berrino et al. 2005). Increased serum testosterone levels in breast cancer cases vs controls were more pronounced in postmenopausal women compared with premenopausal women (Table 1). However, the association between increased androgen levels and breast cancer risk has not been observed in all studies (Hankinson et al. 1998, Adly et al. 2006), with breast cancer risk being ameliorated if the levels of serum oestrogens were taken into account (Table 1), suggesting that the conversion of testosterone into E₂ by aromatase activity contributes to the association between testosterone and breast cancer risk (see sections ‘Limitations in the measurement and interpretation of serum testosterone levels and breast cancer risk’ and ‘Intracrine androgen metabolism in the breast’). Additionally, no association has been observed between serum levels of steroids, including testosterone, and the risk of developing ductal carcinoma in situ (DCIS), a putative breast cancer precursor lesion (Zeleniuch-Jacquotte et al. 2005).

Exogenous androgens

The link between breast cancer development and androgen treatment has been investigated in patients prescribed androgens for therapeutic purposes. Studies evaluating
breast cancer risk in postmenopausal women treated with androgens alone for hypoactive sexual disorder, or testosterone in combination with combination oestrogen–progestin hormone replacement therapy (HRT) for treatment of menopausal symptoms, have not definitively indicated an increased risk of breast cancer associated with androgen treatment (reviewed by Braunstein (2007), Krapf & Simon (2009) and Davis (2010)). However, many of these studies had a relatively short follow-up period (<4 years), which may not be sufficient to observe a change in breast cancer development. Studies at the cellular level show a reduced proliferative index in tissues from women given HRT with added testosterone compared with HRT alone, which suggests a protective effect of androgens in normal breast tissue (Hofling et al. 2007). Similarly, a reduced proliferative index was observed in normal human breast tissues cultured ex vivo when exposed to the most potent androgen, DHT (Eigeliene et al. 2012, Ochnik et al. 2014), further supporting a growth inhibitory effect of androgens.

The effect of exogenous androgen treatments on breast tissue has also been studied in transsexuals undergoing gender reassignment. No increase in breast cancer risk has been reported in male to female (MtoF) or female to male (FtoM) transsexuals compared with the general population (Asscheman et al. 2011). However, the findings in FtoM cases should be interpreted with caution given that gender reassignment surgery, including mastectomy, usually occurs relatively soon (<3 years) after commencing hormone treatment. While this limits the utility of the data from FtoM transsexuals with regard to breast cancer risk, the mastectomy tissue from these individuals

<p>| Table 1  | Association between serum testosterone levels and breast cancer risk in prospective studies |
|-----------------|----------------------------------|-----------------------------------|-------------------------------|-------------------|</p>
<table>
<thead>
<tr>
<th>Reference</th>
<th>Cases</th>
<th>Controls</th>
<th>Subgroups</th>
<th>Testosterone levels in cases as a percent of control (P value)</th>
<th>Hazard or risk ratio (range)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premenopausal women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zeleniuch-Jacquotte et al. (2012)a</td>
<td>274</td>
<td>683</td>
<td></td>
<td>108 (0.01)</td>
<td>1.70 (1.10–2.70)</td>
<td>0.06</td>
</tr>
<tr>
<td>Dorgan et al. (2010)a</td>
<td>98</td>
<td>168</td>
<td>Follicular</td>
<td>113 (0.005)</td>
<td>2.90 (1.40–6.30)</td>
<td>0.005</td>
</tr>
<tr>
<td>Wang et al. (2009)</td>
<td>110</td>
<td>110</td>
<td>Luteal</td>
<td>92 (0.235)</td>
<td>0.77 (0.40–1.50)</td>
<td>0.387</td>
</tr>
<tr>
<td>Eliassen et al. (2006)a</td>
<td>190</td>
<td>374</td>
<td>Luteal</td>
<td>96 (0.501)</td>
<td>1.07 (0.55–2.06)</td>
<td>0.352</td>
</tr>
<tr>
<td>Kaaks et al. (2005a,b)</td>
<td>370</td>
<td>726</td>
<td>Luteal</td>
<td>100 (0.11)</td>
<td>1.80 (0.90–3.40)</td>
<td>0.35b</td>
</tr>
<tr>
<td>Sturgeon et al. (2004)</td>
<td>160</td>
<td>174</td>
<td>Follicular</td>
<td>103 (0.09)</td>
<td>2.01 (1.10–3.60)</td>
<td>0.05b</td>
</tr>
<tr>
<td>Micheli et al. (2004)a</td>
<td>65</td>
<td>243</td>
<td>Luteal</td>
<td>108 (0.01)</td>
<td>1.73 (1.16–2.57)</td>
<td>0.01</td>
</tr>
<tr>
<td>Yu et al. (2003)</td>
<td>127</td>
<td>130</td>
<td></td>
<td>96 (NG)</td>
<td>0.92 (0.50–1.80)</td>
<td>NS</td>
</tr>
<tr>
<td>Thomas et al. (1997)</td>
<td>68</td>
<td>182</td>
<td></td>
<td>NG (NG)</td>
<td>2.16 (0.60–7.61)</td>
<td>0.28</td>
</tr>
<tr>
<td>Postmenopausal women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fourkala et al. (2012)a</td>
<td>200</td>
<td>400</td>
<td>ERz negative</td>
<td>125 (0.04)</td>
<td>2.15 (1.26–3.17)</td>
<td>0.0006b</td>
</tr>
<tr>
<td>Farhat et al. (2011)c</td>
<td>111</td>
<td>594</td>
<td>ERz positive</td>
<td>94 (0.16)</td>
<td>0.58 (0.33–1.02)</td>
<td>0.08b</td>
</tr>
<tr>
<td>Baglietto et al. (2010)</td>
<td>857</td>
<td>197</td>
<td></td>
<td>116 (0.004)</td>
<td>1.68 (1.04–2.07)</td>
<td>0.005c</td>
</tr>
<tr>
<td>Sieri et al. (2009)a</td>
<td>165</td>
<td>672</td>
<td></td>
<td>100 (NG)</td>
<td>1.25 (0.78–2.01)</td>
<td>0.37c</td>
</tr>
<tr>
<td>Wang et al. (2009)</td>
<td>139</td>
<td>138</td>
<td></td>
<td>112 (0.006)</td>
<td>3.13 (1.86–5.28)</td>
<td>&lt;0.001c,d</td>
</tr>
<tr>
<td>Kaaks et al. (2005a,b)a</td>
<td>677</td>
<td>1309</td>
<td></td>
<td>121 (0.029)</td>
<td>2.93 (1.57–5.49)</td>
<td>0.047b</td>
</tr>
<tr>
<td>Zeleniuch-Jacquotte et al. (2004)</td>
<td>297</td>
<td>563</td>
<td></td>
<td>110 (&lt;0.001)</td>
<td>1.85 (1.33–2.57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Missmer et al. (2004)</td>
<td>628</td>
<td>312</td>
<td></td>
<td>110 (0.002)</td>
<td>2.15 (1.29–3.59)</td>
<td>0.005c</td>
</tr>
<tr>
<td>Yu et al. (2003)</td>
<td>170</td>
<td>171</td>
<td></td>
<td>115 (&lt;0.001)</td>
<td>1.40 (0.90–2.20)</td>
<td>0.003b</td>
</tr>
<tr>
<td>Marjor et al. (2003)</td>
<td>134</td>
<td>438</td>
<td></td>
<td>122 (0.002)</td>
<td>2.08 (1.06–4.09)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Key et al. (2002)f</td>
<td>633</td>
<td>1765</td>
<td></td>
<td>108–110NG (NG)</td>
<td>1.48 (0.88–2.49)</td>
<td>0.4b</td>
</tr>
</tbody>
</table>

Bold italics indicate significance (P < 0.05). Hazard or risk ratios and CIs given represent the difference between the lowest and the highest brackets of serum testosterone. Data on additional androgenic and oestrogenic serum steroids can be found in Supplementary Table S1. Unless otherwise stated all risk/hazard values are from non-adjusted risk models.

aFree testosterone was also assessed, see Supplementary Table S1.

*These studies examined the effect of adjusting for E2 levels on the risk associated with serum testosterone levels. In Eliassen, Fourkala, Farhat (ERz-negative cohort) and Marjor, there was no effect observed with E2 adjustment whereas in Farhat (ERz-positive cohort), Wang, Zeleniuch-Jacquotte (2004), Mismer and Key, if the levels of risk associated with testosterone were adjusted for E2, the significance of testosterone levels diminished.

Only bioavailable (adjusted for SHBG concentrations) testosterone levels were reported.

When divided into ERs-positive and ERs-negative subtypes only the ERs-positive subtype remains significant.

Two cohorts with testosterone levels given separately.

Reanalysis of nine previous studies.
provides a unique opportunity to examine the effects of long-term androgen treatment on breast tissue morphology, cellular proliferation and gene expression. In these tissues, no morphological changes associated with breast cancer risk were observed (Burgess & Shousha 1993, Shufelt & Braunstein 2008). While microarray studies examining gene expression profiles have indicated that there are some common immune-, stroma- and epithelial-related gene expression signatures present in both FtoM breast tissues and malignant breast tissue, the functional significance of these pathways to breast carcinogenesis is yet to be determined (Bentz et al. 2010). Breast cancer cases have been reported in MtoF transsexuals, but they are extremely rare (Mueller & Gooren 2008, Asscheman et al. 2011). This suggests that prior exposure to male levels of circulating androgens earlier in life may potentially protect against breast cancer development later in life, although further studies with a larger sample size are required to confirm this observation.

**Limitations in the measurement and interpretation of serum testosterone levels and breast cancer risk**

As outlined above, studies to date have not provided consistent data on the association between testosterone and breast cancer risk. This is largely due to the limitations in the techniques used for measurement of testosterone and the difficulties associated with their interpretation. Most importantly, the conversion of testosterone to E2 by the aromatase enzyme in breast tissue is unaccounted for in the majority of studies investigating testosterone and breast cancer risk (reviewed by Shufelt & Braunstein 2008). Therefore, it is not clear as to whether circulating testosterone is a risk factor _per se_ or a surrogate for increased local production of E2, which directly drives tumour growth. Given the complex intracrine metabolism of steroids that takes place in breast tissue (see section ‘Intracrine androgen metabolism in the breast’), levels of serum androgens are a relatively crude predictor of intracellular androgen action, and tissue levels of androgen may have more prognostic value, although this is yet to be explored. In addition, a number of study design factors may explain the discrepancies between reports examining serum testosterone levels and breast cancer risk. Sample size probably contributes to contradictions regarding the significance of androgens in determining breast cancer risk as larger studies have more statistical power to detect smaller differences as significant (Yu et al. 2003, Sturgeon et al. 2004, Kaaks et al. 2005a, Eliassen et al. 2006). The methodology used for measuring testosterone is also important as at any one time, only a small percentage (~2%) of circulating testosterone is free and available for uptake into cells (Rothman et al. 2011), while the remainder is sequestered to SHBG and biologically unavailable for cellular uptake and receptor binding. To date, all large-scale studies have used immunoassays to measure serum testosterone levels, which have known limitations in sensitivity and specificity (Harwood & Handelsman 2009). Mass spectrometry is emerging as a more accurate method for measuring testosterone compared with immunoassays, and the use of this methodology may potentially provide clearer information regarding testosterone and breast cancer risk. Immunoassays also do not directly measure levels of free testosterone, which is instead measured indirectly through calculations based on independent measurements of total testosterone and SHBG; the accuracy of this method is under debate (de Ronde et al. 2006, Sartorius et al. 2009). In some studies (Missmer et al. 2004, Zeleniuch-Jacquotte et al. 2012), this calculation has strengthened the significance of the risk association between testosterone and breast cancer (Supplementary Table S1 and references therein). Another complicating factor in premenopausal women is the variation in testosterone levels across the menstrual cycle; mass spectrometry analyses show that there is a statistically significant increase in testosterone levels at the mid-cycle corresponding to the ovulatory peak of E2 from the ovaries (Rothman et al. 2011, Bui et al. 2013). While this intra-individual cyclic difference in testosterone is small in comparison to the variation in testosterone observed at a population level (Braunstein et al. 2011), one study has suggested that the mid-cycle peak may demonstrate an association of testosterone and breast cancer risk that is not observed at other stages (Eliassen et al. 2006).

**Prognostic value of AR expression in breast cancer**

The AR, the intracellular receptor that mediates the biological effects of androgens, has been detected in up to 85% of primary breast cancers and up to 75% of metastatic lesions (Lea et al. 2001, Park et al. 2010, Honma et al. 2013), although as discussed below, this frequency varies between different breast cancer subtypes. The majority of studies investigating the relationship between AR levels in the primary tumour, clinical characteristics and disease outcome have found that AR expression is a favourable prognostic indicator (reviewed by Hickey et al. 2012). In cohorts not selected on the basis of estrogen receptor-α (ERα (ESR1)) status,
AR positivity has been associated with longer relapse-free, metastasis-free and overall survival, smaller tumour size and lower histological grade (Bryan et al. 1984, Langer et al. 1990, Kuenen-Boumeester et al. 1992, Soreide et al. 1992, Schippinger et al. 2006, Agrawal et al. 2008, Gonzalez et al. 2008, Hanley et al. 2008, Ogawa et al. 2008, Soiland et al. 2008, Park et al. 2010, Yu et al. 2011, Peters et al. 2012, Honma et al. 2013). High AR levels have also been associated with a favourable response to chemotherapy and hormonal therapy, including anti-oestrogens, aromatase inhibitors and progestins (Teulings et al. 1980, Bryan et al. 1984, Birrell et al. 1995, Agrawal et al. 2008, Chintamani et al. 2010, Champlakorn et al. 2011, Loibl et al. 2011). Importantly, AR has also been shown to be an independent predictor of relapse-free, metastasis-free and/or overall survival in some studies (Soreide et al. 1992, Gonzalez et al. 2008, Soiland et al. 2008, Yu et al. 2011). However, other studies have shown that AR levels do not predict the response to endocrine therapy or relapse-free or overall survival (Allegra et al. 1979, Soreide et al. 1992, Carreno et al. 2007). Discrepancies in study results could be explained by multiple factors, including the following: i) the use of tissue microarrays vs whole-tissue sections; ii) use of different AR protein detection techniques (radioligand binding, immunohistochemistry (IHC) or reverse-phase protein array); iii) use of different antibodies for AR immunodetection; iv) measurement of AR mRNA via different platforms in transcriptome profiling studies; v) use of different cut-off scores to define AR positivity and vi) differences in clinical parameters such as sample size, prior treatment regimens, length of follow-up and tumour type. Two recent meta-analyses have reported that AR expression is associated with better breast cancer outcomes (Qu et al. 2013, Vera-Badillo et al. 2014). However, numerous shortcomings associated with these analyses, most notably the exclusion of several key studies, limit their use in further clarifying the prognostic value of AR.

Given the high degree of heterogeneity in breast cancer, the role of AR should be considered in the context of the different subtypes of disease. In the general diagnostic setting, IHC analysis of ERz, progesterone receptor (PR (PGR)) and human epidermal growth factor receptor 2 (HER2 (ERBB2)) in tumour samples is routinely performed to categorise tumours as ERz-positive, HER2-overexpressing or triple-negative (ERz-negative, PR-negative and HER2-negative) tumours. This enables selection of patients for anti-oestrogen or anti-HER2 therapies. Recently, gene expression profiling and other bioinformatic approaches performed in a research setting have identified a more complex array of breast cancer molecular subtypes, including luminal A, luminal B, HER2, molecular apocrine (luminal AR, LAR) and basal (Perou et al. 2000, Sorlie et al. 2001, Farmer et al. 2005, Parker et al. 2009, Lehmann et al. 2011, Perou 2011, Curtis et al. 2012), which can predict prognosis and will facilitate the development of new opportunities for more personalised therapies. Studies examining the prognostic value of AR expression in specific breast cancer subtypes are described below.

**ERz-positive breast cancer**

ERz-positive cancers are currently defined by the detection of ERz in at least 1% of cells within the tumour (Hammond et al. 2010) and make up ~75% of all breast cancers. ERz-positive cancers are more likely to be AR positive compared with other types of breast cancer (Gonzalez-Angulo et al. 2009, Peters et al. 2009, Luo et al. 2010, Micello et al. 2010, Niemeier et al. 2010, Park et al. 2010, 2011, Hu et al. 2011, Loibl et al. 2011, Yu et al. 2011). In the majority of studies, AR expression in ERz-positive tumours has been associated with favourable characteristics such as improved relapse-free survival, overall survival, response to endocrine treatments and chemotherapy, older age at diagnosis, lower tumour grade, lower Ki67 positivity, smaller tumour size and less necrosis (Gonzalez-Angulo et al. 2009, Peters et al. 2009, Castellano et al. 2010, Niemeier et al. 2010, Hu et al. 2011, Park et al. 2011, 2012, Honma et al. 2013, Witzel et al. 2013). AR expression was also associated with improved overall survival for women with ERz-positive breast cancers in both meta-analysis studies (Qu et al. 2013, Vera-Badillo et al. 2014). In some of these studies, multivariate analyses have demonstrated that AR is an independent predictor of reduced risk of relapse and/or death (Gonzalez-Angulo et al. 2009, Peters et al. 2009, Castellano et al. 2010, Niemeier et al. 2010, Hu et al. 2011, Park et al. 2011, 2012). A prognostic index incorporating scores for AR positivity (>1%), small tumour size and lack of lymph node involvement has been used to predict good prognosis in ERz-positive cancers (Castellano et al. 2013).

ERz-positive tumours can be further classified into luminal A and luminal B subtypes and some studies have specifically examined AR in these cancers. AR expression has been reported to be highest in luminal-type cancers, with luminal A cancers expressing AR more frequently than luminal B (Collins et al. 2011, Yu et al. 2011). Nevertheless, AR expression in both luminal A and luminal B cancers is associated with a reduced risk of relapse and longer survival (Yu et al. 2011). In luminal B cancers, AR positivity has been associated with increased time to relapse and disease-specific survival (Castellano et al. 2010).
Despite this apparently positive role for AR in ERα-positive breast cancers, some studies suggest that high AR expression may be detrimental in tamoxifen-treated breast cancers, potentially contributing to tamoxifen resistance. There is some evidence that tamoxifen-resistant tumours express higher levels of AR than tamoxifen-sensitive tumours (De Amicis et al. 2010), while a high ratio of AR in comparison to ERα has recently been associated with an increased risk of failure of tamoxifen therapy (Cochrane et al. 2014). These findings suggest that AR may have context-dependent roles in ERα-positive breast cancer that can be influenced by prior hormonal therapies.

The generally favourable prognostic indication associated with AR expression in many studies of ERα-positive breast cancers is consistent with the predominantly inhibitory effect of androgen treatment on proliferation of ERα-positive breast cancer cell lines. The main cell line models used to study ERα-positive breast cancer have been the MCF7, ZR-75-1 and T-47D cell lines, which have a luminal molecular phenotype and express AR to some degree (Neve et al. 2006, Kao et al. 2011). Numerous studies have shown that androgens consistently inhibit basal and oestrogen-induced proliferation of the ZR-75-1 and T-47D breast cancer cell lines (Poulin et al. 1988, Birrell et al. 1995, Lapointe et al. 1999, Ortmann et al. 2002, Cops et al. 2008). The effects of androgen treatment in the MCF7 cell line are more variable, with reports of androgens both stimulating and inhibiting proliferation (reviewed by Hickey et al. (2012)). This may relate to differences in AR levels with passage number of the cells, culture conditions that influence AR expression or the variation in steroid receptor levels that is documented in different sub-lines of MCF7 (Horwitz et al. 1978). Furthermore, the ratio of AR:ERα expression can influence the response to androgens; increasing the AR:ERα ratio through overexpression of AR inhibits E2-induced proliferation in T-47D and MCF7 breast cancer cells (Szelei et al. 1997, Peters et al. 2009). The molecular interactions between AR and ERα are discussed further in section ‘AR and ERα’.

ERα-negative breast cancer

ERα-negative breast cancers comprise ~30% of all breast cancers and are typically more aggressive and have poor survival prognoses compared with ERα-positive tumours (Barcellos-Hoff 2013). AR expression in ERα-negative breast cancer is correlated with a lower Nottingham grade and apocrine differentiation (Niemeyer et al. 2010). The association between AR and survival is not so clear in ERα-negative breast cancers as in ERα-positive breast cancers; studies have reported either no association (Gonzalez et al. 2008, Peters et al. 2009, Hu et al. 2011, Park et al. 2011) or an association with improved survival (Agoff et al. 2003, Luo et al. 2010, Witzel et al. 2013). In addition, one meta-analysis reported that AR is associated with improved overall survival in ERα-negative breast cancer (Qu et al. 2013), while the other reported no association (Vera-Badillo et al. 2014). A possible explanation for these contradictory results is that ERα-negative cancers display a high degree of histological and molecular heterogeneity, necessitating larger cohort numbers to determine a role for AR in specific subtypes of ERα-negative disease. Studies examining AR in specific ERα-negative breast cancer subtypes are discussed below.

Molecular apocrine breast cancer

The molecular apocrine subtype is characterised by high expression of AR and a gene expression profile that closely resembles that of luminal breast cancer, despite being ERα-negative (Farmer et al. 2005, Doane et al. 2006, Iggo 2011). While breast cancers defined as molecular apocrine tend to be enriched for HER2+ tumours, not all display this feature. Molecular apocrine tumours that lack HER2+ are probably similar to the LAR subtype of triple-negative breast cancers (TNBCs) (Lehmann et al. 2011). No studies have examined the prognostic significance of AR levels specifically in molecular apocrine breast cancers, in part because these tumours represent ~12% of all breast cancers and such analyses would necessitate large cohorts of unselected cases. However, molecular apocrine cancers are highly aggressive and have a poor prognosis, comparable to that of basal cancers (Lehmann-Che et al. 2013). Given that AR expression is a defining feature of molecular apocrine breast cancers, it is feasible that AR may, at least in part, contribute to poor prognosis in this subtype. This concept is supported by in vitro studies that demonstrate that AR signalling promotes proliferation of cell line models of molecular apocrine breast cancer. To date, the MDA-MB-453 cell line has been the most widely used model of AR-positive, ERα-negative breast cancer and has been classified as molecular apocrine (or LAR) by gene expression profiling (Doane et al. 2006, Lehmann et al. 2011). Proliferation of MDA-MB-453 cells is stimulated by androgens (Hall et al. 1994, Birrell et al. 1995, Doane et al. 2006, Ni et al. 2011) and inhibited by AR antagonists or AR silencing (Birrell et al. 1995, Lehmann et al. 2011, Robinson et al. 2011). The AR cistrome in MDA-MB-453 cells has been shown to be more similar to that of ERα in MCF7 cells than AR in LNCaP prostate cancer cells (Robinson et al. 2011). This suggests that AR may act as a
surrogate ERx in molecular apocrine breast cancer, potentially explaining its oncogenic role in this breast cancer subtype. These studies have formed the basis for trials of the AR antagonist bicalutamide (NCT00468715) and the new generation compound enzalutamide (NCT01889238 and NCT02091960) as therapy for ERx-negative, AR-positive metastatic breast cancer. However, a mutation in the ligand-binding domain of ER (NCT01889238 and NCT02091960) as therapy for and the new generation compound enzalutamide (NCT00468715) may explain its oncogenic role in this breast activity in response to androgens (Moore et al. 2012). This may limit the utility of this cell line as a model of molecular apocrine breast cancer, necessitating analysis of AR function in additional pre-clinical models of this disease subtype to inform development of suitable therapeutic strategies. Proliferation of other TNBC cell lines classified as LAR, including SUM185PE and CAL-148, has also been shown to be induced by androgen signalling and inhibited by the AR antagonist bicalutamide (Lehmann et al. 2011, Ni et al. 2011). However, the MFM-223 cell line is also classified as LAR, but unlike MDA-MB-453 cells it is inhibited by androgen and not sensitive to bicalutamide, although AR knockdown reduces colony formation (Hackenberg et al. 1991, Lehmann et al. 2011). The divergent proliferative effects of androgens on different in vitro models of AR-positive ERx-negative breast cancer probably reflects the heterogeneity of ERx-negative disease and suggests that not all ERx-negative AR-positive breast cancers will benefit from therapies that inhibit AR activity. This is further supported by a clinical trial in ERx-negative, AR-positive metastatic TNBCs, for which the clinical benefit rate for bicalutamide was only 19% (Gucalp et al. 2013). Therefore, AR expression is not sufficient to identify patients suitable for AR-targeting therapies and characterisation of additional markers of favourable response to these therapies is required.

HER2+ breast cancer The HER2+ subtype is enriched for tumours that overexpress HER2 (via gene amplification or dysregulation) and the majority (~70%) are ERx negative (Prat & Perou 2011). HER2-overexpressing tumours are more likely to express AR than other ERx-negative subtypes (Micello et al. 2010, Niemeier et al. 2010, Park et al. 2011), suggesting an association between HER2 and AR in these cancers, although this is not observed in all studies (Kollara et al. 2001, Park et al. 2010). There is a growing body of evidence suggesting that crosstalk between AR and HER2 signalling is an important growth regulatory mechanism in ERx-negative breast cancer (see section ‘AR, FOXA1 and HER2’). Despite this, AR expression is not associated with survival in HER2-overexpressing ERx-negative breast cancers (Gonzalez-Angulo et al. 2009, Micello et al. 2010, Yu et al. 2011), indicating the deficiencies in our understanding of the role of AR in this disease subtype.

Triple-negative breast cancer TNBCs generally have a very low frequency of AR expression compared with other breast cancer subtypes, with as few as 12% of TNBC patients expressing AR (Gonzalez-Angulo et al. 2009, Luo et al. 2010, Niemeier et al. 2010, Park et al. 2010, 2011, Loibl et al. 2011, Yu et al. 2011, Qi et al. 2012, Tsutsumi 2012, Gucalp et al. 2013). In some studies, AR does not predict survival in TNBCs (Gonzalez-Angulo et al. 2009, Micello et al. 2010, McNamara et al. 2013a). However, in other studies, AR expression in TNBCs is associated with a better disease-free and overall survival as well as other favourable tumour characteristics such as lower grade, lower mitotic score, less frequent metastasis and tumour recurrence (Rakha et al. 2007, Loibl et al. 2011, He et al. 2012, Mrklic et al. 2012, Tang et al. 2012, McNamara et al. 2013a,b) as well as improved response to some chemotherapeutic agents in vitro (Koo et al. 2009). Three studies specifically investigating TNBCs were included in the meta-analysis, which showed that AR expression was associated with worse overall survival but had no significant effect on disease-free survival (Qu et al. 2013). These studies demonstrate that the prognostic significance of AR in TNBCs is yet to be clarified.

Basal breast cancer Basal breast cancers, which account for ~90% of TNBCs, are often ERx negative, PR negative and lack HER2 amplification; however, gene expression profiling demonstrates that basal breast cancers and TNBCs are not synonymous (Cleator et al. 2007, Badve et al. 2011). The frequency of AR positivity is lowest in basal breast cancers (Collins et al. 2011), where AR expression is associated with a reduced risk of relapse and longer survival (Yu et al. 2011). A recent study has suggested that unlike the non-basal TNBCs, there is no association between AR and lower Ki67 index in AR-expressing basal-like TNBCs (McNamara et al. 2014), indicating the need to better understand AR action in this subtype.

Androgen-regulated pathways in breast cancer

Recent studies investigating androgen action in breast cancer cell lines have identified several key proteins and signalling pathways that can modulate AR activity and/or mediate the phenotypic outcomes of AR action, including...
FOXA1, PI3K/AKT/MAPK, PTEN, p53 and cell cycle regulators. Interestingly, some of these proteins have been identified as the most frequently dysregulated pathways in breast cancers when analysed by multiple profiling platforms (Cancer Genome Atlas 2012). In addition to these signalling networks, AR also functionally interacts with the ERα and HER2 signalling pathways to regulate breast cancer cells. A summary of these interactions, including how they crosstalk with each other in addition to AR, and the impact this has on proliferation, is depicted in Fig. 1.

AR and ERα

AR and ERα signalling crosstalk has been demonstrated in multiple luminal breast cancer cell lines and this mediates the inhibitory effect of AR signalling on proliferation of ERα-positive breast cancer cells (see section ‘ERα positive breast cancer’). In particular, ligand-activated AR, or overexpression of constitutively active AR, has been shown to inhibit ERα activity in ZR-75-1, T-47D and MCF7 breast cancer cell lines (Lapointe et al. 1999, Panet-Raymond et al. 2000, Ando et al. 2002, Peters et al. 2009, Need et al. 2012). This inhibitory effect of AR on ERα activity requires the DNA-binding domain of the AR, and in part appears to involve competition with ERα for binding to regulatory regions of ERα target genes (Fig. 1A; Peters et al. 2009, Need et al. 2012). These findings provide mechanistic insight into the importance of the AR:ERα expression ratio in the regulation of breast cancer cell proliferation and clinical outcome (see section ‘ERα positive breast cancer’) (Ando et al. 2002, Peters et al. 2009, Cochrane et al. 2014). Interestingly, the steroid receptor co-activator ARA70 (NCOA4) is influenced by the AR:ERα ratio; in the presence of low levels of androgens, ARA70 enhances oestrogen-dependent gene expression in breast cancer).
MCF7 cells; however, upon AR overexpression, ARA70 interacts with AR to antagonise ERα signalling (Fig. 1A; Lanzino et al. 2005). This suggests that coactivators play an important role in mediating the phenotypic outcomes of cells with different AR:ERα ratios.

**AR, FOXA1 and HER2**

FOXA1, a member of the forkhead family of pioneer factors, is a major regulator of DNA binding capacity for ERα and AR in breast and prostate cancer cells, respectively (Augello et al. 2011, Robinson & Carroll 2012). Recent studies have also highlighted the role of FOXA1 in mediating AR DNA binding in ERα-negative breast cancer cells (Ni et al. 2011, Robinson et al. 2011). In the MDA-MB-453 molecular apocrine breast cancer cell line, there is a high overlap of FOXA1 and AR DNA-binding sites, particularly near androgen-regulated genes (Ni et al. 2011, Robinson et al. 2011). Furthermore, FOXA1 ablation reduces MDA-MB-453 colony formation (Robinson et al. 2011) and blocks androgen induction of the oncogenic transcription factor MYC (Ni et al. 2013). In the absence of AR activation in an ERα-negative breast cancer context, FOXA1 interacts with TCF7L2 on DNA to repress AR target genes, whereas upon hormone stimulation, AR replaces TCF7L2 in the FOXA1 complex, leading to induction of gene transcription (Fig. 1B; Ni et al. 2013).

In ERα-negative MDA-MB-453 cells, androgens induce expression of WNT7B and HER3, leading to activation of the Wnt/β-catenin and HER2 signalling pathways (Fig. 1B), which are required for androgen-induced growth of these cells (Ni et al. 2011). DHT-induced HER2 signalling also induces PI3K/AKT pathway activation, in turn leading to phosphorylation and degradation of MAD1 (MXD1), which disrupts the MAD1–MAX complex (Ni et al. 2013). This allows MAX to interact with MYC, also directly induced by DHT via an AR–FOXA1 interaction (Fig. 1B), leading to enhanced oncogenic function of MYC (Ni et al. 2013). Consistent with the functional interactions between FOXA1 and HER2, FOXA1 is more frequently expressed in HER2-amplified breast cancers compared with HER2-negative breast cancers, and HER2 signalling results in the induction of FOXA1 expression (Naderi et al. 2012).

Androgens also enhance activation of ERK, a downstream effector of HER2, and HER2–AR crosstalk is required for proliferation and viability of molecular apocrine breast cancer cell lines (Naderi & Hughes-Davies 2008, Chia et al. 2011, Naderi et al. 2011), a concept that is supported by bioinformatic analysis of gene expression profiles in molecular apocrine breast cancer tissues (Sanga et al. 2009). This reveals that AR and FOXA1 utilise a complex regulatory network to enhance the tumourigenic potential of breast cancer cells, at least in a molecular apocrine context.

In ERα-positive ZR-75-1 breast cancer cells, FOXA1 binding sites collocate with AR-binding sites, although to an extent lesser than that observed for ERα (Need et al. 2012). Little, however, is known about the importance of FOXA1 for AR DNA binding in an ERα-positive context. AR signalling generally has a suppressive effect on cell growth in ERα-positive AR-positive breast cancer cells, and studies to date have associated FOXA1 with mitogenic effects of both AR and ERα in prostate and breast cancer cells. Therefore, a potential role for FOXA1 in mediating the protective effects of AR on ERα-positive breast cancer is not well defined.

**AR, PTEN, p53 and PI3K/AKT**

In ERα-negative MDA-MB-453 cells, DHT induces PTEN, a tumour suppressor that inhibits PI3K enzymes (Mester & Eng 2013), via AR binding to an androgen-responsive element in the PTEN upstream promoter (Fig. 1C; Wang et al. 2011). AR-mediated up-regulation of PTEN in breast cancer may form part of a closed feedback loop where PTEN represses PI3K action, which in turn reduces AR activity (Fig. 1C). Interestingly, PTEN shares a bidirectional promoter with another tumour suppressor, KLLN, which is also up-regulated by androgens in ERα-negative MDA-MB-453 and ERα-positive MCF7 cell lines (Wang et al. 2013a,b). Downstream KLLN actions include induction of p53 (TP53) and p73 (TP73), which promote apoptosis (Fig. 1C). In interpreting these results, it is worth noting that these authors (Wang et al. 2013a,b) report an anti-proliferative effect of androgen treatment in these cell lines, in contrast to a number of other studies that report proliferative effects of androgen (Hackenberg et al. 1988, 1993, Maggiolini et al. 1999, Birrell et al. 1995, Ni et al. 2011, Robinson et al. 2011). Although the reasons for these discrepancies are unknown, this indicates the potential for dualistic effects of AR signalling in MDA-MB-453 and MCF7 breast cancer cell lines. Furthermore, induction of these tumour suppressor genes by DHT is consistent with the anti-proliferative effects of DHT observed by these authors in these cell lines (Wang et al. 2013a,b) and provides mechanistic insight into the genes and pathways that mediate the proliferative effects of androgens in breast cancer. DHT induced p53 expression in MCF7 breast cancer cells (Wang et al. 2013a,b), while AR expression has been associated with a lack of p53 expression in unselected...
human breast cancers (Ogawa et al. 2008). However, in a different cohort that was separated into ERα-positive or ERα-negative disease, no association between AR and p53 was observed in either group (Agoff et al. 2003). Therefore, while there is some evidence to support the functional interactions among AR, PTEN and p53, further studies are required to clarify the role of this pathway in breast cancer cells.

Additional studies support a functional association between AR and the PI3K/AKT pathway in breast cancer. PI3K/AKT signalling regulates a number of downstream mitogenic pathways in breast cancer, and tumours with PI3K (PIK3CA) mutations often express high levels of AR (Gonzalez-Angulo et al. 2009). Breast cancer cell lines that express AR may be more sensitive to the PI3K inhibitor NVP-BEZ235 than AR-negative lines; however, only ERα-positive AR-positive lines such as MCF7 and BT-474 demonstrated enhanced inhibitory effects of NVP-BEZ235 in the presence of DHT (Lehmann et al. 2011, Wang et al. 2013a,b). This is perhaps due to the latter study reporting growth inhibitory effects rather than growth stimulatory effects of DHT in ERα-negative AR-positive lines such as MDA-MB-453. However, combined inhibitory effects of AR antagonists and PI3K inhibition in this context have not been reported to date. This suggests that the crosstalk between ligand-dependent AR signalling and the PI3K pathway can regulate growth of breast cancer cells.

AR and cell cycle regulators

The breast cancer susceptibility gene and cell cycle regulatory protein BRCA1 has been shown to interact with the AR and synergise with the coactivator GRIP1 (NCOA2) to enhance AR activity on a transfected AR-responsive probasin reporter gene in ERα-positive MCF7 and ERα-negative HBL-100 breast cancer cells (Park et al. 2000). Mutations in the BRCA1 protein have been shown to be associated with a lower prevalence of AR immunoreactivity in breast cancer tissues (Berns et al. 2003, Pristauz et al. 2010), further suggesting a link between AR and the tumour suppressive role of BRCA1 in breast tissues. Additional evidence for AR modulation of cell cycle pathways is derived from studies demonstrating that AR expression is positively correlated with that of RB1 (Bieche et al. 2001). Cyclin D1 (CCND1), which controls entry into the S phase of the cell cycle, is also regulated by AR; recruitment of ligand-bound AR in combination with the orphan nuclear receptor DAX1 to an androgen-responsive element in the CCND1 promoter results in repression of the transcription of CCND1 and subsequent inhibition of proliferation in ERα-positive MCF7 breast cancer cells (Fig. 1D; Lanzino et al. 2010). Similarly, studies on ERα-negative MDA-MB-231 cells stably transfected with AR, which are growth inhibited by androgen, indicate that anti-proliferative effects are mediated through a decrease in cyclin D1 levels and an increase in the cyclin-dependent kinase (Cdk) inhibitor p21 (CDKN1A), which is required for the proliferative effects of androgen (Fig. 1D; Garay et al. 2012). Importantly, inhibition of proliferation in this model required activation of both AR and the EGFR/MAPK signalling pathway, whereas activation of either of these pathways alone led to increased proliferation (Garay et al. 2012). This indicates the potential importance of the MAPK pathway in mediating the proliferative effects of androgens, although this requirement for MAPK has not been studied in the context of breast cancer cells expressing endogenous AR.

Intracrine androgen metabolism in the breast

Intracrinology refers to the study of steroid hormone synthesis and metabolism within the cells or tissues where their actions are exerted and without transport of hormones through serum, a process that efficiently directs the most potent hormones to the cells that need them (Labrie 1991). In humans, the majority of biologically active androgens and oestrogens in the body are produced locally in target tissues from circulating adrenal and/or ovarian precursors. Differences in intracrine pathways could also result in individuals with similar serum androgen levels having marked differences in tissue androgen levels. This has the potential to confound interpretation of studies investigating androgens in breast cancer, especially those investigating breast cancer risk associated with circulating androgens. Furthermore, as intracrine pathways regulate levels of androgen, and therefore AR activity, within breast cancer cells, the potential for mis-interpretation also exists for studies investigating the prognostic value of AR in breast cancer or the intracellular mechanisms of AR action in breast cancer cells in vitro. It is therefore critical to elucidate the metabolic pathways that modulate intracellular levels of androgens in the breast, and how these may be dysregulated in breast cancer. A complex system of enzymes regulates androgen and oestrogen levels in breast cells (Fig. 2), and the enzymes that have been implicated in breast cancer are described below.

Aromatase

Aromatase catalyses the process of conversion of the androgen, testosterone, to the most potent oestrogen,
E2 (Fig. 2), which generally stimulates proliferation of breast epithelial cells. This forms the basis for aromatase inhibition as a therapeutic strategy for breast cancer, which is now a standard first-line treatment regimen for postmenopausal patients with ERα-positive disease (Santen et al. 2009, Sasano et al. 2009). While aromatase inhibition decreases E2 levels, testosterone levels increase in the breast tissue, which may contribute to the efficacy of aromatase inhibitors in ERα-positive disease through the anti-proliferative effect of androgens (Macedo et al. 2006, Chanplakorn et al. 2011). It has been proposed that any findings of increased breast cancer risk associated with endogenous serum testosterone are largely due to conversion to E2 by aromatase in the breast (reviewed by Shufelt & Braunstein (2008)). Therefore, in studies evaluating a potential association between serum testosterone and breast cancer risk, it is necessary to determine whether there is a direct risk from circulating testosterone, or an indirect risk from the metabolism of testosterone to E2 by aromatase. As shown in Table 1, several studies have addressed this and some found that the increased breast cancer risk associated with increased serum testosterone is ameliorated when testosterone to E2 conversion is taken into account. Although these studies only measured serum levels of hormones and not the potential for localised synthesis of E2 from circulating testosterone by aromatase in breast tissues, they do suggest that aromatase activity may be an important modifier of breast cancer risk.

Further direct interactions between androgen signalling and aromatase have been suggested at the molecular level. A recent study has described a novel mechanism where activated AR co-operates with the orphan nuclear receptor DAX1 to suppress ERα-mediated transcription of aromatase (Lanzino et al. 2013). Additionally, the androgen-regulated microRNA Let-7a (hsa-let7a) (Lyu et al. 2014) clusters with Let-7f (hsa-let-7f), which has been shown to suppress...
aromatase in ERα-positive MCF7 breast cancer cells (Shibahara et al. 2012). Therefore, the protective actions of androgens, at least in ERα-positive breast cancers, may be partially achieved through down-regulation of aromatase and decreased intracrine synthesis of oestrogens.

5α reductase family

5α reductase (5αR) enzymes catalyse the conversion of testosterone to DHT (Fig. 2). This prevents E2 synthesis, as DHT is not a substrate for aromatase, and acts as an amplification of the androgen signal due to the higher affinity of DHT compared with testosterone for the AR. Of the three known isoforms, 5αR1 is expressed more frequently in the breast than 5αR2 and is considered the principal isoform (Suzuki et al. 2001) despite its lower affinity for testosterone (Jin & Penning 2006), while 5αR3 has not been reported in the breast. The importance of 5αR1 in breast cancer development remains unclear, as either higher (Lewis et al. 2004) or lower (Zhao et al. 2010) levels of 5αR1 have been reported in tumour compared with matched normal tissues. However, a large number of studies suggest an inhibitory role for 5αR1 in disease progression. In invasive ductal carcinoma, 5αR1 expression is inversely correlated with tumour grade and proliferative index (Suzuki et al. 2006, McNamara et al. 2013a), decreased in invasive carcinomas compared with organ-confined disease (Shibuya et al. 2008, McNamara et al. 2014) and decreased in metastatic lymph nodes compared with primary tumours (Shibahara et al. 2013). Additionally, tumours positive for both AR and 5αR1 are smaller in size, exhibit reduced Ki67 proliferative index and are associated with longer disease-free and overall survival compared with tumours that express either one or neither of these markers (Suzuki et al. 2006, McNamara et al. 2013a, 2014). 5αR1 has also been shown to be up-regulated by androgen treatment in the MDA-MB-453 breast cancer cell line, suggesting a mechanistic relationship between AR and 5αR1 (McNamara et al. 2014). Importantly, this suggests that expression of androgen-metabolising enzymes can strengthen the prognostic value of AR, particularly in TNBCs that do not exhibit a clear association between AR and clinical outcome (see section ‘Triple negative breast cancer’). This effect on prognosis may be due to androgen regulation of steroid intracrinology, indicating the need for a better understanding of androgen intracrinology to better define the role of AR in breast tumorigenesis.

Polymorphisms in the 5αR2 gene that decrease enzyme activity, and therefore reduce DHT levels, are associated with an increased breast cancer risk (Francis et al. 2014) and/or shorter survival (Bharaj et al. 2000, Scorilas et al. 2001), although significant harmful effects of these alleles have not been consistently reported (Spurdle et al. 2001, Yang et al. 2002, van Gils et al. 2003). Increased levels of 5αR2, but not 5αR1, were detected in ERα-positive breast tumours in which the proliferation index decreased by more than 40% following aromatase inhibitor therapy (Chaplakorn et al. 2011). These studies suggest that while 5αR2 may not be the predominant isoform, it nevertheless may play an important protective role in breast cancer in addition to 5αR1.

17β-hydroxysteroid dehydrogenase type 5 family

17β-hydroxysteroid dehydrogenases (17βHSDs) catalyse many reactions, including the reversible conversions between A4 and testosterone and between E1 and E2 (Fig. 2), thus controlling the balance between the potent steroids testosterone and E2, and their less potent counterparts A4 and E1. The principal 17βHSD enzyme studied with regard to androgen action in breast tissues is 17βHSD5 (AKR1C3), which catalyses the conversion between A4 and testosterone (Dufort et al. 1999, Nagasaki et al. 2009, Suzuki et al. 2010). At present, 17βHSD5 expression in cancer as opposed to normal tissues is uncertain, with some studies suggesting a decrease and others an increase in cancer compared with normal tissues (Vihko et al. 2005, Han et al. 2008, Byrns & Penning 2009). Correlations between 17βHSD5 expression and other clinico-pathological markers such as age, stage and tumour size have not been observed (Suzuki et al. 2005, Han et al. 2008, McNamara et al. 2013a). However, expression of 17βHSD5 does correlate with that of 5αR1 and AR in both ERα-positive and TNBCs (Suzuki et al. 2001, McNamara et al. 2013a, 2014) and is lost in lymph node metastasis in ERα-positive breast cancers (Shibahara et al. 2013) and between DCIS and IDC in triple-negative cancers (McNamara et al. 2014). In interpreting the significance of 17βHSD5 expression and androgen metabolism in breast epithelial cells, a confounding factor is that substrates for 17βHSD enzymes are not limited to androgenic steroids; other substrates include progestagen and progesterone (Byrns & Penning 2009, Byrns et al. 2010). Consequently, it is necessary to determine both enzyme and substrate levels in breast tissue to accurately assess the importance of 17βHSD5 activity in testosterone metabolism. However, co-expression of 17βHSD5 with other androgen biosynthetic enzymes in precursor lesions and breast cancers suggests that it is probably an important factor in the regulation of androgen action in this disease.
3α- and 3β-hydroxysteroid dehydrogenase enzymes

The potent androgen DHT is metabolised by 3α- and 3β-hydroxysteroid dehydrogenase enzymes (3αHSD and 3βHSD) to 3α-diol and 3β-diol respectively (Fig. 2). While little is known about the expression of these enzymes in breast cancer, 3αHSD2 is expressed in the mammary gland (Penning et al. 2000). 3β-diol has been shown to bind ERα and stimulate proliferation in ERα-positive T-47D and ZR-75-1 breast cancer cell lines (Chen et al. 2013), demonstrating that these androgen metabolites can have weak oestrogenic properties, thus enhancing ERα function while decreasing AR activity through metabolism of DHT. In breast cancer tissues, presence of the most potent oestrogen, E2, may minimise the biological relevance of these weakly oestrogenic metabolites to breast cancer biology (Honma et al. 2011). However, in an oestrogen-deplete environment, such as in postmenopausal women or women undergoing aromatase inhibitor therapy, 3α-diol and 3β-diol may exert a proliferative effect on breast epithelial cells.

Steroid sulphatase and UDP glucuronosyltransferase enzymes

Comparatively, few studies have investigated the role of steroid-deconjugating or -conjugating enzymes such as steroid sulphatase (STS) or UDP glucuronosyltransferase (UGT) in relation to androgens in breast cancer. STS acts on substrates such as DHEAS, oestradiol-S and oestrone-S, deconjugating the sulphate group from the steroid backbone and thus generating more potent steroid derivatives (Fig. 2). The localised production of active androgens from DHEAS is particularly relevant in postmenopausal women, in whom the majority of serum steroids derived from the adrenals are circulating in a hydrophilic sulphated form. To date, the role of STS in breast cancer has been investigated more in relation to the production of oestrogens rather than androgens (reviewed by McNamara et al. (2013b)). An increase in the levels of STS has been shown to be associated with poor outcomes in breast cancer (Utsumi et al. 1999, Suzuki et al. 2003, Chanplakorn et al. 2010), suggesting that inhibition of this enzyme is a potential therapeutic strategy (Geisler et al. 2011). Indeed, increased STS enzyme activity is postulated as a potential mechanism for the failure of endocrine therapy for breast cancer due to an associated increase in oestrogen levels (Chanplakorn et al. 2010). Whether or not expression of STS in breast cancers alters androgen levels is not known. Similarly, little is known about the androgen-conjugating UGT enzyme family that glucuronidates and inactivates lipophilic compounds, including androgens and oestrogens (Fig. 2). UGT enzymes are expressed in the breast (reviewed by Mackenzie et al. (2010)) and have been shown to be induced by androgens in MCF7 and MDA-MB-453 breast cancer cell lines (Harrington et al. 2006, Moore et al. 2012). There is evidence that a variant allele ((TA)N/(TA)N) of the UGT family member, UGT1A1, is associated with a reduced risk of ERα-negative breast cancer compared with ERα-positive breast cancer (Sparks et al. 2004). In addition, reduced expression of UGT2B7, UGT2B10 and UGT2B11 is observed in highly dense normal breast tissues, an established breast cancer risk factor, compared with less-dense tissues (Haakensen et al. 2010), suggesting that these enzymes can potentially contribute to breast tumorigenesis. However, whether or not changes in UGT affect breast tissue due to alterations in serum or tissue androgen or oestrogen levels is unknown. The inactivation/catabolism of androgens is an important but understudied aspect of androgen action in the breast.

Conclusion/future directions

There is ample evidence that androgens and the AR signalling pathway are attractive therapeutic targets for breast cancer. While AR agonists such as testosterone propionate and fluoxymesterone acetate were used successfully to treat breast cancer until the 1980s, AR antagonists are now being investigated as potential new therapies. However, a better understanding of AR action in different breast cancer subtypes is still required to effectively exploit this pathway for clinical benefit. Current clinical trials investigating AR antagonism in breast cancer patients have focused on ERα-negative AR-positive disease, but trials are now being proposed for ERα-positive breast cancer, where the role of AR is probably quite different. Identification of appropriate markers of AR responsiveness in different breast cancer contexts is essential to better select patients who will benefit most from AR antagonist therapies. Additionally, there has been little consideration to date regarding the use of selective AR modulators (SARMS) as therapeutic agents to selectively activate AR signalling in ERα-positive disease without the masculinising side effects associated with traditional AR agonist therapies. Recent results from a phase II clinical trial of the SARM enobosarm (NCT01616758) demonstrate the clinical benefit in six out of 17 postmenopausal women with ERα-positive metastatic breast cancer, suggesting that this is a promising new therapeutic approach (Overmoyer et al. 2014).

http://erc.endocrinology-journals.org  DOI: 10.1530/ERC-14-0243  © 2014 Society for Endocrinology  Published by Bioscientifica Ltd.  Printed in Great Britain

Downloaded from Bioscientifica.com at 12/19/2018 01:43:04AM  via free access
Given the predominant inhibitory role of androgen signalling in ERα-positive breast cancer, this approach may be more successful. Again, the biomarkers of the response will be critical for patient selection.

In addition to the agents directly targeting the AR, there is potential for the manipulation of other components of the AR signalling pathway to modulate the effects of androgens in breast cancer. While detailed molecular mechanisms of AR action in the breast are still being elucidated, it is apparent that AR interacts with several critical signalling pathways implicated in breast carcinogenesis. Therapeutic strategies indirectly targeting AR by modulating interacting factors, such as androgen-metabolising enzymes, the FOXA1 pioneer factor or cell cycle regulators, are either in use (e.g. the 5αR inhibitor finasteride or the CYP17A1 inhibitor abiraterone acetate) or under pre-clinical investigation (e.g. Cdk inhibitors) in prostate cancer, and similar approaches may prove clinically useful in breast cancer. In fact, trials for abiraterone acetate (e.g. NCT01842321, NCT00755885 and NCT01381874), which suppresses androgen production, and Cdk inhibitors (e.g. NCT01864746 and NCT01624441) are in progress for breast cancer. Recent results have indicated that addition of abiraterone acetate to the aromatase inhibitor exemestane did not improve the outcome for postmenopausal patients with ERα-positive breast cancer (O’Shaughnessy et al. 2014, trial NCT01381874), suggesting that inhibition of AR signalling is not a suitable approach in this disease subtype. However, abiraterone therapy may be more beneficial for women with ERα-negative breast cancer, which is also currently under investigation (NCT01842321 and NCT00755885). While it is interesting to use information from studies on other hormone-dependent cancers, such as prostate cancer, as a potential indication of AR-interacting proteins in the breast, it is also important to remember that these pathways are potentially disease state specific and/or influenced by other therapies in vivo.

Finally, although this review has focused on the actions of AR in breast cancer, we do not fully understand the role of AR in normal breast tissue, either developmentally or with regard to changes associated with menstrual cycles, pregnancy and lactation. In primates, AR is expressed in all stages of mammary development except for late pregnancy and lactation and appears to show small, non-significant variation across the menstrual cycle (Cheng et al. 2005). Ar knockout transgenic models generally indicate that AR is required for normal mammary gland development (reviewed by Hickey et al. 2012, Chang et al. 2013 and Tarulli et al. 2014). In the human, AR appears to have a protective role in normal breast at least in part by opposing ERα action (Dimitrakakis et al. 2003, Eigeliene et al. 2012, Ochnik et al. 2014). This raises the possibility of SARMs being used for breast cancer chemoprevention strategies in women with a high breast cancer risk. In support of this, a recent study has reported that AR expression, along with ERα and vitamin D receptor, forms a signature to classify normal breast cells and co-expression of these three receptors can also predict better breast cancer outcome (Santagata et al. 2014).

In conclusion, androgen and AR actions are highly complex in breast cancer. Their role in the disease varies depending on the expression of ERα and other interacting pathways. As inhibitory therapies targeting AR have been established for prostate cancer, AR is an attractive ‘low-hanging fruit’ in the treatment of breast cancer. However, refining selection criteria and determining ‘in whom’ and ‘to do what’ will be essential in the effective clinical utilisation of agents that either specifically inhibit or selectively activate AR to improve the breast cancer outcomes.

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/ERC-14-0243.

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 funded by grants from the National Health and Medical Research Council of Australia (ID 1008349 to W D Tilley), Cancer Australia (ID 1043491 to W D Tilley, T E Hickey and N L Moore), the National Breast Cancer Foundation of Australia (ID NC-08-12 to T E Hickey and NC-12-21 to N L Moore) and Susan G Komen for the Cure (ID BCTR0601118 and BCTR0504475 to W D Tilley). T E Hickey holds a Postdoctoral Fellowship Award from the U.S. Department of Defense Breast Cancer Research Program (W81XWH-11-1-0592) and K M McNamara was supported for part of this work by an Australian Academy of Science/Japan Society for the Promotion of Science postdoctoral fellowship.

Author contribution statement
K M McNamara and N L Moore wrote and prepared the manuscript and prepared the figures and tables. T E Hickey, H Sasano and W D Tilley contributed to manuscript editing and critical review.

Acknowledgements
The authors wish to thank Dr Gerard Tarulli for helpful discussions regarding the manuscript.
References


Fertility and Sterility *77* Suppl 4 S3–SS. (doi:10.1016/S0015-0282(02)02985-0)


Hackenberg R, Hawighorst T, Filmer A, Nia AH & Schulz KD 1993 Medroxyprogesterone acetate inhibits the proliferation of estrogen- and progesterone-receptor-negative MFM-223 human mammary


Jin Y & Penning TM 2006 Multiple steps determine the overall rate of the reduction of 5α-dihydrotestosterone catalyzed by human type 3 3α-hydroxysteroid dehydrogenase: implications for the elimination of androgens. *Biochemistry* **45** 13054–13063. (doi:10.1021/bi060591r)


Endocrine-Related Cancer


Received in final form 18 June 2014
Accepted 19 June 2014
Made available online as an Accepted Preprint
20 June 2014