A review of estrogen receptor/androgen receptor genomics in male breast cancer

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Abstract

Male breast cancer is a rare disease, of which little is known. In contrast to female breast cancer, the very vast majority of all cases are positive for estrogen receptor alpha (ER α), implicating the function of this steroid hormone receptor in tumor development and progression. Consequently, adjuvant treatment of male breast cancer revolves around inhibition of ER α . In addition, the androgen receptor (AR) gradually receives more attention as a relevant novel target in breast cancer treatment. Importantly, the rationale of treatment decision making is strongly based on parallels with female breast cancer. Yet, prognostic indicators are not necessarily the same in breast cancer between both genders, complicating translatability of knowledge developed in female breast cancer toward male patients. Even though ER α and AR are expressed both in female and male disease, are the genomic functions of both steroid hormone receptors conserved between genders? Recent studies have reported on mutational and epigenetic similarities and differences between male and female breast cancer, further suggesting that some features are strongly conserved between the two diseases, whereas others are not. This review critically discusses the recent developments in the study of male breast cancer in relation to ER α and AR action and highlights the potential future studies to further elucidate the genomic regulation of this rare disease.

Key Words

- male breast cancer
- estrogen receptor
- androgen receptor
- genomics
- endocrine treatment

Endocrine-Related Cancer
(2017) **24**, R27–R34

Introduction

Male breast cancer is rare, accounting for around 1% of all breast cancers (Siegel *et al.* 2016) and is frequently positive for ER α (91–95%) and/or PR (80–81%) (Giordano *et al.* 2004, Anderson *et al.* 2010, Nilsson *et al.* 2013). The most well-known anti-estrogenic therapy is tamoxifen, which competitively blocks estradiol binding to the receptor, effectively inducing an alternative conformation of ER α ligand-binding domain (Shiau *et al.* 1998), which prevents the formation of an active transcription complex (Zwart *et al.* 2007) and inhibiting the onset of tumor cell proliferation programs (Severson *et al.* 2016). Aromatase inhibitors are also used in the treatment of ER α -positive

breast cancer to prevent the conversion of testosterone to estrogen, thus depleting the patient and therefore the tumor of estrogens, and blocking ER α -driven cell proliferation (Zimniski *et al.* 1993). Both tamoxifen and AIs are prescribed in the adjuvant treatment of female breast cancer (Baum *et al.* 2002, Regan *et al.* 2011). In male breast cancers, tamoxifen (Aisner *et al.* 1979) and aromatase inhibitors (Doyen *et al.* 2010) are both effective.

Androgen receptor (AR) is also frequently positive in both male and female breast cancers (Isola 1993, Peters *et al.* 2009, Hu *et al.* 2011, Chia *et al.* 2015). In ER α -positive female breast cancer, AR is practically always

expressed (Wang *et al.* 2014, Sas-Korczynska *et al.* 2015) and has been linked to differential outcome (Jiang *et al.* 2016). Interestingly, ER α -negative female breast cancers can express AR as well, in about 8–12% of the cases, termed molecular apocrine breast cancer (Farmer *et al.* 2005, Doane *et al.* 2006, Lakis *et al.* 2014). Gene expression profiles in ER α -/AR+ breast cancers are similar to ER α + /AR+ breast cancers (Doane *et al.* 2006), suggesting that AR may compensate in the absence of ER α . These results are further confirmed by ChIP-seq analyses, which revealed a potential of AR to compensate for the lack of ER α expression by binding the same genomic regions to regulate expression of many of the same genes (Robinson *et al.* 2011).

Recently, multiple reports shed further light on the genomics, transcriptomics and epigenetic gene regulation of male breast cancer. Furthermore, clinical trials have been initiated to specifically block ER α and/or AR in male breast cancer. These new observations together with parallels as well as differences with the female disease call for a review paper to update us on the most recent developments in the field of male breast cancer in relation to genomic features and novel therapeutics.

Clinical features

Although the development of the male breast is distinctly different from that of the female breast, male breast cancers are typically treated in the same fashion as female breast cancers in post-menopausal women (Korde *et al.* 2010). The underlying biology of the vast majority of male breast cancers is thought to be fully dependent on endocrine stimulation as both ER α and AR are typically expressed in these tumors (Hong *et al.* 2016). The majority of circulating estrogens in men (~80%) originate from peripheral aromatization of adrenal and testicular androgens. The remaining ~20% comes from direct production in the testes (Doyen *et al.* 2010). Administration of monotherapy aromatase inhibitors is not advised because of the risk of secondary resistance due to a likely feedback mechanism allowing for additional substrate (testicular androgens) for aromatization, which has been associated with increased estradiol levels at progression (Doyen *et al.* 2010). It has been suggested AIs could be more effective in combination with a pituitary gonadotropic-releasing hormone (GnRH) analogue to block this feedback loop leading to an efficacious suppression of estrogen production (Sousa *et al.* 2013).

Because of the rarity of breast cancer in males, there are few clinical trials involving exclusively male breast cancer patients making it difficult to determine the benefit of particular treatments. Tamoxifen is effective in

male breast cancer patients, but recurrence does occur at a similar frequency as compared to female breast cancer (Kiluk *et al.* 2011). Although tamoxifen is a beneficial treatment, it is not without clinical issues including side effects such as reduced libido and impotence (Arnould *et al.* 2006), which may lead to a clinical or patient decision to stop treatment (Fentiman *et al.* 2006). In addition, the prognostic indicators are not necessarily the same between male and female breast cancers, making it difficult to determine which patients truly require treatment (Abreu *et al.* 2016, Leone *et al.* 2016).

AR is known for many years as driver of tumorigenesis in prostate cancer via receptor activation by ligand binding and subsequent downstream expression of genes critically involved in tumor cell proliferation (Brinkmann *et al.* 1999, Xu *et al.* 2006, Wang *et al.* 2009, Ikonen & Mills 2012). Function of AR and AR-mediated gene expression are evolutionary related to ER biology (Mangelsdorf *et al.* 1995, Shang *et al.* 2002, Wang *et al.* 2005, 2007). Current therapies to block AR action in prostate cancer are aimed to inhibit testosterone production by abiraterone (Li *et al.* 2015), prevent the nuclear import of the receptor (enzalutamide (Tran *et al.* 2009)) and/or block coactivator recruitment (bicalutamide (Schellhammer 2002)). These novel therapies have successfully been used in primary and metastatic prostate cancers (Perlmutter & Lepor 2007, Zhao *et al.* 2016b). Recently, the selective antagonist apalutamide has been developed to bind AR thus rendering it unable to bind cofactors and inhibiting downstream effects (Clegg *et al.* 2012). As breast cancer expresses AR as well, these existing therapeutics that were originally designed for the treatment of prostate cancer are considered promising regimens in the management of breast cancer. Consequently, several clinical trials examining the efficacy of AR inhibition alone or in combination with other treatments such as anti-estrogen therapy (Table 1) are currently ongoing in both female and male breast cancer.

Molecular features

Although the paucity of male breast cancer patients makes it a difficult disease to study, recent work from several groups has undertaken the molecular characterization of male breast cancer. Breast cancer is well described at the transcriptional and (epi)genomic levels. Molecular characteristics are known to differ between male and female breast cancers (e.g. *BRCA2* germline mutation frequency (Johannson *et al.* 2014, Silvestri *et al.* 2016)). This indicates additional molecular

Table 1 Current running clinical trials (clinicaltrials.gov) examining androgen receptor inhibition in breast cancer.

Study	Patient group (gender)	Trial and treatment setting	Treatment	Androgen inhibition	Estrogen inhibition
NCT02676986	ER+/TN-AR+ (female)	Phase II, neoadjuvant	Enzalutamide monotherapy ± exemestane	Yes	Yes
NCT02353988	TN-AR+ (female)	Phase II, metastatic	Bicalutamide monotherapy	Yes	No
NCT01990209	AR+ (both)	Phase II, metastatic	Orteronel monotherapy	Yes	No
NCT00468715	AR+/ER-/PR- (both)	Phase II, metastatic	Bicalutamide monotherapy	Yes	No
NCT02348281	TN-AR+ (female)	Phase II, metastatic	Bicalutamide monotherapy	Yes	No
NCT02605486	AR+ (female)	Phase I/II, metastatic	Palbociclib + bicalutamide	Yes	No
NCT01638247	ER+ and/or PR+ (male)	Phase II, adjuvant, neoadjuvant or palliative	Tamoxifen ± GnRH analogue vs AI ± GnRH analogue	No	Yes
NCT02457910	TN-AR+ (both)	Phase Ib/II, metastatic	Taselib + enzalutamide OR enzalutamide monotherapy	Yes	No
NCT02689427	TN-AR+ breast cancer (both)	Phase IIb, neoadjuvant	Enzalutamide + paclitaxel	Yes	No
NCT02368691	TN-AR+ (female)	Phase II, metastatic	Enobosarm monotherapy	Yes	No
NCT01889238	TN-AR+ (female)	Phase II, metastatic	Enzalutamide monotherapy	Yes	No
NCT01616758	ER+ (female)	Phase II, metastatic	Enobosarm	Yes	No
NCT02750358	TN-AR+	Phase II, adjuvant	Enzalutamide monotherapy	Yes	No
NCT00755885	ER+ (female)	Phase I/II, metastatic	Abiraterone acetate	Yes	No
NCT02007512	ER+ and/or PR+ (female)	Phase II, metastatic	Enzalutamide + exemestane	Yes	Yes
NCT02437318	ER+ and/or PR+ (both)	Phase II, metastatic	Alpelisib ± fulvestrant	No	Yes
NCT01597193	ER+/AR+ (both)	Phase I, incurable	Enzalutamide ± anastrozole, exemestane or fulvestrant	Yes	Yes
NCT02091960	AR+/HER2+ (female)	Phase II, metastatic	Enzalutamide ± trastuzumab	Yes	No
NCT01842321	Molecular apocrine/HER2-(female)	Phase II, metastatic	Abiraterone acetate	Yes	No
NCT01517802	ER+ (female)	Phase II, metastatic	Abiraterone acetate + prednisone/ prednisolone ± exemestane	Yes	Yes

features may differentiate between genders, potentially leading to additional or new treatments for male breast cancer. The vast majority of male breast cancers express AR (Murphy *et al.* 2006, Shaaban *et al.* 2012), and the receptor is considered a valid drug target in this setting, as evidenced by recent initiation of multiple clinical trials (Table 1). FBC trials are also listed in this table for reference. In ER α -positive breast cancer cells, AR has been shown to limit ER α -mediated growth (Cops *et al.* 2008, Peters *et al.* 2009) indicating the propensity to confer better response in patients who are positive for both receptors. This finding is supported in MBC by clinical work in which ER α -positive/AR-positive patients had significantly better outcome compared to ER α -positive/AR-negative patients, and their hormone receptor immunohistochemical profiles illustrated co-clustering based on ER (both ER α and ER β) and AR expression (Shaaban *et al.* 2012). In FBC on the other hand, ER α clustered with progesterone receptor, whereas AR clustered with ER β , suggesting an intrinsic difference in hormone receptor biology and hormone receptor dependencies between genders. Specifically in MBC, AR has been shown to be a valid target for treatment in the metastatic setting (Di Lauro *et al.* 2015).

To add complexity to the role of AR in breast cancer, preclinical studies have demonstrated AR may have both proliferative (De Amicis *et al.* 2010) and anti-proliferative (Szelei *et al.* 1997, Andò *et al.* 2002, Cops *et al.* 2008, Peters *et al.* 2009) properties. Not surprisingly, as these receptors are transcription factors with broad effects on transcription, differential binding at these sites can be indicative of outcome in both breast and prostate cancer. Further research is needed to characterize the binding sites of AR in both male and female breast cancer so the responsive genes can better be determined from a more functional standpoint, leading to increased knowledge of the action of AR in breast cancer and its likely weak points, which may be exploited therapeutically.

In contrast to male disease, female rates of ER α -and/or PR-positivity are higher (Giordano *et al.* 2004, Anderson *et al.* 2010, Nilsson *et al.* 2013). Notably, AR is highly expressed in male breast cancers (Zhou *et al.* 2014) and has been found to be indicative of good outcome in male breast (Shaaban *et al.* 2012) and poor outcome in hormone-refractory prostate cancer (Buhmeida *et al.* 2006). These discordant results may be indicative of inherent differences between breast and prostate cancer or they may highlight the importance of treatment setting

in these studies as ER α /AR dual positivity has recently been shown to be associated with improved outcome (Li *et al.* 2016).

A recent study by Johansson and coworkers examining the gene expression profiles of MBC identified two novel subgroups using unsupervised hierarchical clustering, that are associated with different clinical and biological features (Johansson *et al.* 2012). These two subgroups of MBC, the luminal M1 and luminal M2 subgroups, do not completely overlap with FBC luminal A/B classifications suggesting potential ER α -related differences in MBC, which may be clinically relevant. As gene expression is typically related to protein expression, examination of the differences between MBC and FBC were studied at the HR immunohistochemical level in a large series of 514 matched cases (Shaaban *et al.* 2012). Strikingly, hierarchical clustering revealed in FBC ER α clustered together with PR, whereas in MBC, ER α clustered with AR suggesting a clinically actionable difference between genders in hormone receptor biology.

The frequency of *BRCA2* mutations is notably higher in MBC than FBC (Rizzolo *et al.* 2013, Silvestri *et al.* 2016). In a recent study, Biesma and coworkers found 22% of MBC samples investigated (15/69) had a *BRCA2*-like profile based on DNA copy number data indicating a significant proportion of MBCs may be susceptible to PARP inhibition therapy or specific chemotherapy regimens aimed at DNA double-strand breaks (Biesma *et al.* 2015). In addition, with the exception of gains on chromosome X in MBC, this work found few differences between MBC and FBC (luminal-like) with respect to copy number changes (Biesma *et al.* 2015). This is a particularly interesting finding as AR is found on chromosome X and may therefore be upregulated in these samples. A recent study of similar size (59 MBC samples) also found that MBCs and FBCs of similar subtype shared many recurrent copy number aberrations (Piscuoglio *et al.* 2016). Unfortunately, this study did not determine *BRCA2*-like status based on copy number profiles despite reporting 5% of the samples as germline *BRCA2* mutated, so validation of high frequency of *BRCA2*-like status in MBC (Biesma *et al.* 2015) and potential AR upregulation could not be investigated.

In an integrative analysis, Johansson and coworkers used an algorithm to detect candidate driver genes for tumorigenesis in MBC by combining copy number and gene expression data in both MBC and FBC samples, which revealed differences in the pathways and processes associated with the drivers between MBC and FBC suggesting differential regulatory processes between the two cancers (Johansson *et al.* 2013). Indeed, an extensive

examination of genome-wide DNA methylation profiles in MBC has indicated there are two major subgroups, ME1 and ME2, which were associated with the gene expression subgroups luminal M1 and luminal M2 (Johansson *et al.* 2015). ME1 tumors were enriched for hypermethylation at polycomb target genes with respect to ME2, with ME1 and its associated gene expression subgroup luminal M1 to be associated with more aggressive tumors. Moreover, this work identified hypermethylated genes in the ME1 subgroup to be associated with androgen response suggesting a possible link to AR function.

To examine further likely driver genes in MBC tumorigenesis, Piscuoglio and coworkers determined the mutational landscape in MBC in relation to FBC. In this study, 59 samples were subjected to detection of somatic mutations for a subset of 241 genes that are frequently recurrently mutated in cancer or involved in DNA repair pathways (Piscuoglio *et al.* 2016). Genes identified as most frequently mutated included *GATA3*, a known interactor of ER α (Theodorou *et al.* 2013) and regulator of luminal cell differentiation associated with ER α -positive FBC breast cancer. Unfortunately, because this work only examined a very small subset of pre-selected genes, the analysis was intrinsically biased without an opportunity to discover new genes, which may be mutated in MBC. Genome-wide sequencing would solve the problem of biased gene selection, but a drawback is that a significant proportion of mutations in DNA do not give rise to functional or stable transcripts (Shah *et al.* 2012). Using RNA-seq of MBC samples can give both genome-wide mutational data and identify mutations that are found in a stable transcript and thus most likely to have a biological consequence on the protein level, lending additional weight to mutational findings.

Although ER α is the main driver of MBC, affecting downstream transcription and tumor cell proliferation, the functionality of ER α in MBC has remained completely unexamined. In FBC, genome-wide ER α /DNA binding profiles determined by chromatin immunoprecipitation coupled with massively parallel sequencing (ChIP-seq) have been found to be dynamic upon tamoxifen exposure (Severson *et al.* 2016) and more importantly are associated with differential outcome (Ross-Innes *et al.* 2012, Jansen *et al.* 2013). H3K27ac signal, a histone modification indicative of active enhancers at ER α -binding regions, is similar between male and female tissues suggesting the inherent transcriptional programming between genders is comparable (ENCODE Project Consortium 2012). As MBC is generally ER α driven, we suggest a similar action of ER α in MBC to FBC, which may also indicate the potential

for outcome determination of male breast cancers on the basis of differential ER α binding. In addition, several groups have profiled AR/DNA binding in prostate cancer and have shown associations of distinct AR signatures with outcome (Stelloo *et al.* 2015, Nevedomskaya *et al.* 2016, Zhao *et al.* 2016a). These findings indicate that these techniques can be applied to breast tumor samples as well, to better characterize the functional binding patterns of both ER α and AR in male breast cancer in relation to patient prognostication.

Discussion

Both in science as well as clinical practice, we tend to couple distinct diseases to genders and specific gene products. Breast cancer is almost automatically linked with female and estrogen receptor alpha (ER α) action. Although this is intrinsically true, it is by far not the full story, as breast cancer is neither gender-exclusive nor fully ER α mediated. Analogous to this, androgen receptor (AR) action is not confined to the prostate alone, but is highly expressed in most breast cancers of both genders. A common feature between male and female breast cancer is that resistance to endocrine therapeutics is frequently observed. As endocrine therapy resistance is clinically identified at the level of recurrent disease, a clear clinical need exists to identify markers for endocrine resistance at an early stage, so that patients with a predicted resistance can be treated with an alternative treatment instead. ER α and AR both have thousands of known DNA-binding sites in breast and prostate cancer, respectively (Carroll *et al.* 2006, Chng *et al.* 2012, Jansen *et al.* 2013, Zwart *et al.* 2013, Stelloo *et al.* 2015, Severson *et al.* 2016). Differential binding at these sites can be indicative of outcome in both breast and prostate cancer. In male breast cancer, such analyses have not been reported to date, and it would be highly relevant to identify biomarkers based on ER α and/or AR behavior in male breast cancers to identify weak points that may be exploited therapeutically.

Although MBC is typically considered ER α driven, there are distinct molecular features found in male breast cancer, which could be used as targets for currently available or yet undeveloped therapies. As most male breast cancers are positive for AR, this steroid hormone receptor could be considered an interesting drug target that may work in synergy with blocking ER action. AR-targeted therapeutics are currently being tested in multiple clinical trials accruing both male and female patients, either as monotherapy or as a combination therapy together with ER α inhibitors. Still, surprisingly

little is known about the biological interaction between ER α and AR in female breast cancer, being completely unexplored in the male setting. Therefore, it remains to be determined whether such treatments will be supported by translational studies. In contrast to female breast cancers, cell line model systems for male breast cancer do not exist, greatly complicating preclinical analyses and the search of novel drug targets in this rare disease. With the recent development of *ex vivo* intervention technologies (Centenera *et al.* 2012, 2013, Dean *et al.* 2012, Ochnik *et al.* 2014), these issues may be resolved in the future, enabling us to effectively bridge biology with clinical practice in a disease as rare as male breast cancer.

Declaration of interest

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

Funding

This work did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Acknowledgments

The authors acknowledge the support of the Netherlands Cancer Institute and the Dutch Cancer Society KWF. They thank Dr Xanthippi Alexi for critically reading the manuscript and valuable suggestions.

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Received in final form 20 December 2016

Accepted 6 January 2017

Accepted Preprint published online 6 January 2017