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Review Article: Androgen receptor and antiandrogen therapy in male breast cancer

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Abstract

Cancers arising in the male breast are uncommon. Male breast cancer is a hormone-driven disease that often expresses the estrogen receptor, and antiestrogen therapy represents the mainstay of treatment. Paradoxically, the advent of a wave of antiestrogens eclipsed the therapeutic potential of alternative therapeutic options. At the beginning of the hormonal therapy era the administration of antiandrogens to metastatic male breast cancer patients was proposed. Ever since the use of these compounds has largely been neglected. A therapeutic role for antiandrogens has been envisioned again in recent years. First, molecular characterization efforts pointed to the androgen receptor as a potential therapeutic target. Second, the development of aromatase inhibitors unexpectedly raised the need for neutralizing androgens in order to tackle endocrine feedback mechanisms responsible for acquired resistance. We herein provide an overview of molecular studies where the androgen receptor was investigated at the genomic, transcriptomic or phenotypic level. We then discuss androgens in the context of the endocrine networks nourishing male breast cancer. Finally, clinical evidence on antiandrogens is summarized along with strategies should be implemented to improve the medical management of these patients.

Keywords

Male breast cancer, androgen receptor, androgens, anti-androgen therapy, gonadotropin-releasing hormone analogues

Introduction

Male breast cancer (MBC) is a rare condition [1]. Even though its incidence is raising with peaks in some African countries, MBC accounts approximately for 0.5-1% of all breast cancer (BC) cases [2, 3]. Owing to the rarity of the disease, obtaining a clear picture of risk factors is tremendously challenging. MBC is a disease of elderly men, as the incidence increases with age without the bimodal pattern present in female BC (FBC), and develops more commonly in men with underlying medical conditions that lead to a high estrogen/androgen ratio like in the case of Klinefelter's syndrome, testicular disorders, obesity or liver diseases [4, 5]. From a genetic perspective, MBC shares some common risk factors with FBC, such as germ-line mutations in BRCA1and BRCA2 [4]. Additional genetic alterations that have been connected with the onset of MBC involve PALB2, androgen receptor (AR), CYP17, CHEK2, and RAD51B [4].

When considering current therapeutic approaches, it is worth mentioning in advance that the evidence that has been collected so far relate to small-sized, retrospective studies. Attempts to provide prospectively-generated data have indeed been frustrated by the difficulties in enrolling participants. The most established therapeutic concept is that MBC is a tumor largely dependent on sex hormones and the oncogenic activities mediated by their cognate receptors [6]. The therapeutic relevance of hormone manipulations is rooted in surgical procedures, such as orchiectomy, adrenalectomy and hypophysectomy, and dates back to 1940s when orchiectomy was first described as an effective treatment for skeletal metastases [2]. These procedures have largely been replaced by hormonal medical treatments. The impressive progress we have witnessed in the medical treatment of hormone receptor-positive FBC has also had an impact on the management of MBC patients. Two interconnected factors explain this. First, most MBCs are estrogen receptor (ER)-positive, and ER is even expressed at a higher frequency than in FBC [7]. Second, a wave of studies, though retrospective in nature, provided clues that ER-directed therapies are effective for treating MBC patients [8-12]. Antitumor efficacy has been reported with virtually all the antiestrogens currently available, namely tamoxifen, aromatase inhibitors (AIs) and fulvestrant [8-12].

Even though, one the one hand, antiestrogen therapy has received significant attention over the past decades, on the other hand its increased success has obscured alternative therapeutic strategies. In the mid-1980s Massimo Lopez theorized similarities between MBC and prostate cancer in terms of androgen dependency, and provided seminal evidence that tumor regressions can be achieved with antiandrogens [13, 14]. Ever since, the therapeutic potential of antiandrogen therapy remained confined to data extrapolated from a few dozen of metastatic MBC patients. The importance of androgens in MBC was paradoxically proposed again in recent years with the advent of AIs. Patients treated with AIs experience an increase of androgen levels, owing to the drop in 17b-estradiol and the consequent activation of the hypothalamic-pituitary feedback loop. This results in an excess of substrate for aromatization that is supposed to oppose the action of AIs [6].

In this article we discuss molecular and endocrine concepts related to the AR and androgens in MBC. We then illustrate available evidence with antiandrogens in the clinical setting, addressing why AR-directed therapies deserve substantially increased consideration.

AR in MBC: genomics

Large initiatives pursuing global molecular characterization of tumors are shedding light on the molecular landscape of the most common malignancies. Tumors arising in a given body site have been reclassified into a number of molecular subtypes [15]. Once data were accumulated, information gathered from multiple layers of molecular characterization (genomics, transcriptomics, proteomics etc.) were integrated [16]. Nowadays, we have a fairly detailed map of the most commonly deregulated pathways and networks coexisting in a given disease entity. The ultimate goal of this impressive functional characterization is twofold: i) matching specific alterations to drugs that selectively switch off aberrantly activated molecular networks, and ii) avoid wasting resources to develop compounds in tumors that are not reliant on the drug target(s). Given the rarity of MBC, neither the molecular interactions driving the disease nor adaptive changes that enable cancer cells to survive stressful conditions (e.g. pharmacological pressure) have been thoroughly investigated. Since discussing molecular alterations in MBC outside those impacting AR signal is not within this review's scope, for a more comprehensive view on this topic the reader may refer to [17]. Briefly, a first wave of studies with characterization purposes provided preliminary evidence on genetic changes and deregulated pathway nodes that operate in MBC. Although they painted an incomplete picture, AR was the focus of early investigations and its druggability has raised expectations. Although the AR abnormalities/antiandrogen therapy pair is intuitive, the evidence is still scattered and functional preclinical studies are missing.

First evidence that MBCs harbour AR mutations dates back to 1992 when a germline mutation in exon 3, encoding the DNA-binding domain, was reported in two brothers with concomitant clinical and endocrine evidence of androgen resistance (Reifenstein syndrome) [18]. One year later, a point mutation in exon 3 was detected after screening 13 MBC patients for the presence of germline mutations in exons encoding the DNA-binding domain [19]. Again, the patient whose tumor carried this AR mutation presented a partial androgen insensitivity syndrome. Since AR-mutant MBC cases were found in an androgen insensitivity context, a protective role for AR was envisioned. The logic behind this was a mutationally-induced decreased activity of AR that nullifies the protective effects of androgens on the male breast. Conversely, it has also been postulated that mutant AR forms might have altered interactions with partner proteins without defective DNA binding ability [20], or that AR mutants gain an altered sequence-specific DNA binding, enabling them to bind to estrogen response elements (EREs) and then promoting the transcription of estrogen-regulated genes [18, 19]. In an endocrine background of elevated estrogen-androgen ratio, like in the case of aged males in whom 17b-estradiol levels are higher than in post-menopausal females [21], this abnormal DNA binding pattern may therefore promote MBC. We cannot rule out alternative possibilities. AR mutations may enhance avidity for androgens, feed promiscuous binding to other ligands, or modify the recruitment and/or balanced activity of co-activators and co-repressors. However, while theories multiplied, the interest surrounding AR mutations was dampened by subsequent case series that failed to provide evidence of germline or somatic mutations [20, 22]. A second chapter that further complicates the picture refers to a highly polymorphic region within the coding area of exon 1, containing a variable number of polyglutamine (CAG) repeats. In the general population this region encodes for 17-26 glutamines [23]. An abnormal expansion of this region is seen in patients with X-linked spinal and bulbar muscular atrophy (Kennedy's syndrome), a condition also characterized by androgen insensitivity

[23]. Conversely, shorter AR polyglutamine tracts have been associated with increased AR activity in preclinical models, and with an increased risk of prostate cancer [24]. The message conveyed is that shorter CAG tracts translate into an increased AR transcriptional activity, whereas longer CAG tracts result in a suboptimal ligand-mediated stimulation of AR. Two studies searching for an association between CAG repeat length and MBC did not notice any appreciable differences between cases and the respective control groups [22, 25]. Two other studies suggested that longer CAG repeats are more common in MBC that in controls [26, 27], and a trend toward a higher frequency of shorter CAG tracts in the control group emerged from a fifth report [28]. Thus, even though genetic evidence is scarce and somewhat ambiguous, the scenario proposed is that androgen hyposensitivity caused by either AR mutations or long CAG repeats might be a causal factor for MBC. If AR emanates protective signals in MBC, how is this connected with tumour regression following exposure to antiandrogens? In interpreting genomic studies on AR it is worthwhile looking at the question from a different angle. Genetic alterations in AR seem extremely rare and possibly define tumors arising in specific syndromic contexts or populations. In a small-sized immunohistochemistry-based study analyzing steroid hormone receptor expression hints of lower mean age at diagnosis for AR-negative tumors were provided [29]. This suggests that the molecular relevance of AR might change with aging, and potentially reconnect with the aforementioned studies. Indeed, two of the three AR-mutated tumors were diagnosed in men younger than 60. From a therapeutic perspective, we argue that the ideal setting to investigate the frequency and therapeutic implications of AR mutations, amplifications, or splice-variant expression is not the basal condition, i.e. at diagnosis in therapy-naïve patients, but rather after disease progression following multiple lines of antihormone treatments. As already established in prostate cancer, AR alterations might indeed arise upon prolonged exposure of cancer cells to a hormone-deprived milieu, representing an acquired event that ensures cell fitness in a hostile environment [30].

AR in MBC: transcriptome-based studies

The first attempts of MBC sub-classification have been recently carried out. At the beginning, genomic profiling of MBC revealed the existence of two subgroups defined as male-complex and male-simple [31]. The latter was designated as a disease occurring exclusively in men. The idea of MBC as a heterogeneous disease was further strengthened upon unsupervised hierarchical clustering of gene expression profiling preformed by the same group [32]. With this approach two distinct subtypes were identified, and defined as luminal M1 (70%) and luminal M2 (30%). The two groups differed both in terms of survival outcomes and underlying biological processes. Even though array data were exclusively interrogated, to our knowledge, for seven gene expression modules and a signature registering the activity of AR was not scrutinized, this study provided further ground to the heterogeneous nature of MBC. An independent gene expression profiling study carried to retrieve differences between MBC and FBC yielded approximately 1.000 differentially expressed genes [33]. Biological interpretation of this gene set revealed that a significant higher number of AR-related genes were up-regulated in MBC compared with FBC, and overall suggested AR activation. Using FBC as a benchmark for the foundation of our reasoning, is it possible to foresee strategies for addressing the molecular consequences of AR activation in MBC and the therapeutic potential of its pharmacological inhibition. In FBC cell lines AR activation elicits opposite outcomes in relation to ER status. In ERα-positive cells androgen treatment exerts

inhibitory effects on ERα-driven proliferation [34]. Conversely, activation of AR signaling promotes proliferation in a subset of ER-negative BC (molecular apocrine or luminal androgen receptor) [35]. Early hints of efficacy were coherently reported with bicalutamide in ER-negative/AR-positive FBC [36], and further supported by results from a phase 2 study with enzalutamide in AR-positive, triple-negative BC patients. In this latter study an androgen-driven gene signature associated with better clinical outcomes was generated [37]. However, in vivo growth inhibition of ER-positive/AR-positive tumors was also seen with enzalutamide, and correlated with a high nuclear AR:ER ratio [35]. In general, the level of segmentation reached by molecular characterization of FBC (for instance, six different molecular entities were described for triple-negative BC [38]) along with the many cellular and animal models available is enabling us to decipher the different scenarios where AR-directed drugs are more likely to be active. Gene expression profiling studies for classification purposes in MBC are in their dawning. Multiplying our efforts to achieve a more granular taxonomy, and establishing cell lines and patient-derived xenografts for functional preclinical studies, are all conditions to be recreated for sharpening the therapeutic potential of AR-targeting agents in MBC.

AR in MBC: expression levels and clinical outcomes

Immunohistochemistry studies of MBC samples reported AR expression in a range of 34 to 95% [39-42]. In addition to this striking variability, controversy exists, and conflicting data were reported, on the association between AR expression and disease stage and/or survival outcomes. In three studies including data from 150 MBC cases, there was no association between AR expression and more advanced disease stage or adverse survival outcomes [39, 42, 43], despite in [39] a negative association between AR expression and MIB-1 scores was found. Conversely, the evaluation of sex hormone receptor status in a series of 39 patients suggested that 5-year disease-free survival and overall survival were significantly shorter for patients with AR-positive tumors [44], as analogously noted in a cohort examining 102 MBC patients where AR-expressing MBC patients apparently derived a lower benefit from tamoxifen [45]. An association between AR expression and longer CAG repeats was also reported negatively impacting survival outcomes [27]. Conversely, in one of the largest series presented so far AR-positive luminal A MBC had improved overall survival compared with matched FBC cases [46]. The same study highlighted gender-related differences in the pattern of expression of sex hormone receptors, potentially underlying non-overlapping steroid receptor interactions. In greater detail, ERα clustered with ERβ isoforms and AR in MBC, whereas it was associated with progesterone receptor and its isoforms in FBC. When considering studies addressing the clinical significance of AR expression, statistically meaningful analyses were hindered, with few exceptions, by the restricted number of cases analyzed. Even the largest series are burdened by their retrospective nature, and partially lack key information related to factors impacting on the explored outcomes, such as (neo)adjuvant therapies. A lack of standardized procedures for assessing AR expression needs to be also considered as a potential confounding factor. Thus, larger investigations exploiting fully annotated clinical series or, alternatively, pooled analyses based on individual patient data are warranted to address the prognostic significance of AR expression in MBC. Finally, a series of interactions deserve to be considered, such as whether the clinical significance of AR expression changes in relation with age at diagnosis, co-expression of other receptors, or administration of adjuvant anti-hormone and/or chemotherapy.

Endocrine concepts supporting therapeutic androgen suppression in MBC

Circulating androgens can feed MBC in two different, partly connected, ways definable as treatment-unrelated and treatment-related [6]. The first case refers to the possibility that circulating androgens, in a condition of no prior exposure to antihormone treatments, directly exert tumor-promoting functions by acting on AR-expressing MBC cells. In aging males the androgen/estrogen ratio shifts in favor of estrogens. This stems from the decrease in testicular and adrenal testosterone production coupled with the age-associated increase in fat mass and the correlated intensified aromatase activity [21]. Although the logic consideration is that estrogens represent the most abundant source of oncogenic stimuli, the concept that androgens elicit the same effects can be deducted from the following: treatment with antiandrogens resulted in tumor shrinkage in a non-negligible fraction of patients [14, 47]. At that time, therapeutic options for metastatic MBC were limited and largely empirical. Consequently, for a given time window we administered AR-directed therapy also in the front-line setting when the hormonal background was not "polluted" by the interference of prior therapies. Even though clinical experience with antiandrogens is mostly confined to the use of cyproterone acetate (CPA), which also possesses antigonadotropic effects, it is unlikely that tumor regression were exclusively due to "off-target" effects. Indeed, a small series suggested that the gonadotropin-releasing hormone (GnRH) analog buserelin had little effects as a monotherapy [48]. Conversely, its concomitant use with the pure antiandrogen flutamide, administered to achieve a maximum androgen blockade, resulted effective.

In a condition of prior exposure to anti-hormone treatments, we now refer to AIs, the action of androgens are dual. Chronic administration of anastrozole to adult male rats provokes an increase in testis weight along with a series of hormonal changes [49]. These include an increase in intratesticular testosterone concentrations together with increased circulating levels of testosterone, follicle-stimulating hormone (FSH) and luteinising hormone (LH). Analogous effects were observed in young male volunteers, elderly men, elderly men with borderline hypogonadism and MBC patients [50-54]. Thus, increased androgen levels during AI therapy represent a sort of "endocrine side effect", influencing MBC biology in two different ways: i) the excess of substrate for aromatization outcompetes the pharmacological effects of AIs, ii) the excess of androgens directly stimulates AR-expressing cancer cells.

These observations support the concept that antiandrogens represent a versatile therapeutic option for advanced/metastatic MBC patients. In a perspective of sequential therapy, they can indeed be integrated in the therapeutic continuum with different purposes and timing.

Clinical studies with antiandrogens

The first report exploring antiandrogens in MBC dates back to 1982 [13]. Administration of CPA in three metastatic MBC showed encouraging signs of antitumor activity. In an expanded clinical series encompassing ten patients published three years later, the use of CPA resulted in seven complete or partial responses [14]. Patients included in this study were mostly pretreated with chemotherapy and/or with hormone manipulations, albeit obsolete, including medroxyprogesterone acetate, fosfoestrol, testolactone or orchiectomy. Even though a decrease in plasma levels of testosterone, estradiol, FSH and LH was observed, there was no association between the magnitude of hormonal suppression and tumor responses. This is not surprising when considering the restricted

number of patients evaluated. The first clues suggesting the usefulness of maximal androgen blockade, achieved by combining the GnRH analogue buserelin with the pure antiandrogen flutamide, were provided in 1988 [48]. In a series of ten men with advanced BC treated in an equal number with buserelin alone or in combination with flutamide, authors reported that only one out of five patients who received the monotherapy derived a benefit, whereas four patients in the combination group experienced a partial remission. Notably, the patient who benefited from buserelin alone had a further response lasting 24 months with the addition of flutamide after recurring bone pain. Another case report describing scan normalization with maximum androgen blockade in a patient with metastatic bone disease was reported in 1990 [55]. Further ground to the combined use of GnRH analogues with an antiandrogen was provided in 1993 [47]. In a series of eleven men the combined use of buserelin with CPA resulted in a clinical benefit (complete or partial response or stable disease) in ten patients. Testosterone, FSH and LH were suppressed to a greater extent than with CPA alone but, again, this did not translate into clear beneficial effects for the patients. By comparing this latest series with that using CPA alone, a suggestion towards better outcomes emerged with the combined approach.

After these seminal reports, the role of antiandrogens remained unexplored for the following twenty years. Only recently, results from an expanded case series analyzing 36 metastatic MBC patients of whom 21 related to [14, 47] have been presented [56]. Fourteen patients were treated with CPA as a monotherapy and 22 with complete androgen blockade. The overall response rate was 52.8%, with a median progression-free survival of 8.9 months and a median overall survival of 24.3 months. More importantly, data on AR expression were available for 7 patients. All the four patients with AR-expressing tumors had a clinical benefit. In one of them the tumor did not express ER, raising the hypothesis that antiandrogen-based therapy should be considered, at least when supported by target expression, for the small fraction of patients with ER-negative tumors. On the other hand, tumor responses were not observed in patients with AR-negative tumors. Thus, for the first time, a predictive significance for AR emerged. Coherently with previous works, some differences, although not statistically significant, in survival outcomes favoring the combination were seen [14, 47]. To address the question regarding the therapeutic relevance of achieving the deepest possible testosterone suppression, we then run a pooled analysis including 60 metastatic MBC patients [57]. Twenty-three men received either CPA or an AI as a monotherapy, whereas for 37 patients a gonadotropin-releasing hormone analogue was added to peripherally-acting agents. We reported nearly significant results favoring the combined treatment for all survival outcomes explored, including median progression-free survival, 1-year progression-free survival rate, median overall survival, and 2-year survival rate. Taken together, the studies discussed above indicate that antiandrogen therapy is endowed with antitumor activity in MBC.

Discussion and future directions

Clinical data available so far suggest that antiandrogens should be considered for patients who already received all potential ER-directed therapies once the disease turned to a refractory form, and in the front-line setting for patients with ER-negative tumors. In principle, their administration should be supported by target expression, although we recognize the difficulties in obtaining this information. The use of GnRH analogues deserves a further mention. The choice of administering a GnRH analogue in combination with an antiandrogen follows the same principles already proposed

for AIs [6, 58]. Therapeutic decisions should be taken on a case-by-case basis, balancing the expected benefit with known toxicities. A sequential approach, envisioning the administration of an antiandrogen as a monotherapy followed by the inclusion of a GnRH analogue after radiological evidence of disease progression, is a strategy that should not be underestimated. This approach is indeed intended to postpone chemotherapy, gaining a therapeutic line. Indeed, we must carefully consider all the harm correlated with chemotherapy in an elderly population, along with the scattered information we have on its safety and efficacy [59]. For instance, it might be considered when there is no need for achieving a quick disease control and a rapid tumor regression.

Finally, how can we exactly capture the efficacy of antiandrogens in MBC? First, it is essential to define the molecular landscape of MBC, both in terms of mutational events and deregulated pathways/biological functions. Even though large initiatives have been deployed (Male Breast Cancer: Understanding the Biology for Improved Patient Care; ClinicalTrials.gov Identifier: NCT01101425), results have not yet been published. From the evidence available, we can only extrapolate that MBC, analogously to more common tumors, is characterized by a certain degree of heterogeneity. However, the full spectrum of disease entities included in the definition of MBC is unknown. Second, preclinical models are necessary for mechanistic studies aimed at elucidating the network in which AR operates, its partners, and the biological output elicited by its activation. Efforts towards realizing a collection of MBC cell lines and animal models, encompassing patient-derived xenografts (tumor fragment-derived and cancer stem cell-based models) and genetically engineered mouse models, will push our understanding forward into the biology of MBC. Finally, from a clinical perspective the following areas should be prioritized. Current evidence with antiandrogens stems from approximately 50 patients treated over a time window of more than three decades. This might confound outcome interpretation. Indeed, techniques and criteria for disease assessment, subsequent therapeutic options, and best supportive care have greatly varied, and have overall improved over time. Collecting novel data and promoting pooled analyses, understanding the relationship existing between AR expression and clinical outcomes, whether or not basal and post-therapy hormonal levels are worth being evaluated in daily clinical practice, are all information required to draw up the identikit of tumors more likely to be dependent on AR stimulation, and then susceptible to androgen deprivation/AR inhibition. Intuitively, increasing the segmentation of a rare disease will add a further level of complexity, especially when we reason about prospective trials. To this end, we recognize the importance of an international cooperation as the basis for delivering better care to our patients.

List of abbreviations

AIs: aromatase inhibitors; AR: androgen receptor; BC: breast cancer; CPA: cyproterone acetate; ER: estrogen receptor; FBC: female breast cancer; FSH: follicle-stimulating hormone; GnRH analogues: gonadotropin-releasing hormone analogues; LH: luteinising hormone; MBC: male

Conflict of interests

The authors declare that they have no competing interests.

Authors' contributions

LDL, MB, RDM and MM-S conceptualized the paper. LP, PV, DS, ADB, MM, VS and DS critically revised drafts of the manuscript. MM-S and MB wrote the manuscript. All authors read and approved the final manuscript.

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