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Commentary

Advances in breast cancer treatment and prevention: preclinical studies on aromatase inhibitors and new selective estrogen receptor modulators (SERMs)

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Abstract

Intensive basic and clinical research over the past 20 years has yielded crucial molecular understanding into how estrogen and the estrogen receptor act to regulate breast cancer and has led to the development of more effective, less toxic, and safer hormonal therapy agents for breast cancer management and prevention. Selective potent aromatase inhibitors are now challenging the hitherto gold standard of hormonal therapy, the selective estrogen-receptor modulator tamoxifen. Furthermore, new selective estrogen-receptor modulators such as arzoxifene, currently under clinical development, offer the possibility of selecting one with a more ideal pharmacological profile for treatment and prevention of breast cancer. Two recent studies in preclinical model systems that evaluate mechanisms of action of these new drugs and suggestions about their optimal clinical use are discussed.

Keywords: aromatase inhibitors, arzoxifene, breast cancer, prevention, rexinoid

Introduction

Hormonal (endocrine) therapy (HT) is one of the most effective treatments for breast cancer in the adjuvant, the metastatic, and the prevention settings. For more than two decades, the anti-estrogen tamoxifen has been the HT of choice for all stages of ER-positive breast cancer [1], and tamoxifen is still the only approved agent, in the United States, to reduce the risk of breast cancer in high-risk women. Tamoxifen is a prototype of a class of drugs called selective estrogen-receptor (ER) modulators (SERMs), which exhibit anti-estrogen effects in the breast but possess estrogen-like activity in other tissues such as bone and blood [1]. This inherent mixed agonist/antagonist nature of tamoxifen is probably responsible for the two major limits of its successful therapeutic promise, i.e. tumor resistance, *de novo* or acquired, seen in many patients, and its adverse effects in other tissues. Developments over the past two decades have led to potentially more effective, less toxic, and safer HT agents that are currently being implemented into the management of

breast cancer, or soon will be. This, in turn, brings the challenge of determining the optimal use of these new drugs, either in combination or in sequence, questions that are currently under investigation in key preclinical models and clinical trials. Two recently reported preclinical studies – one by Long and colleagues [2] that demonstrates the efficacy of anti-estrogens as second-line therapy in breast tumors failing aromatase inhibitor (AI) therapy, and one by Suh and colleagues [3] that shows high synergism between arzoxifene and the new rexinoid LG 100268 in treatment and prevention – make significant contributions in this area and are discussed.

Aromatase inhibitors: clinical efficacy

Estrogen deprivation was suggested long ago as one of the most efficient strategies to block ER action [4]. After menopause, estrogen deprivation is most specifically achieved using inhibitors that block the conversion of adrenal androgens to estrogens by the enzyme aromatase [5]. The third-generation nonsteroidal AIs anastrozole and

AI = aromatase inhibitor; Arz = arzoxifene; ER = estrogen receptor; HT = hormonal therapy; iNOS = inducible nitric oxide synthase; LG268 = LG 100268; NMU = *N*-nitrosomethylurea; SERM = selective ER modulator; TGF- β = transforming growth factor β .

letrozole have both shown, in postmenopausal women, superior efficacy compared with tamoxifen as first-line treatments for advanced breast cancer, and, at least for letrozole, also as neoadjuvant therapy for ER-positive invasive breast cancer (reviewed in [6]). Current results from the ATAC study in postmenopausal women with early ER-positive breast cancer further suggest that in the adjuvant setting, an AI (anastrozole) is superior to tamoxifen in terms of disease-free survival and in preventing contralateral incidents [7]. These AIs may very soon occupy a central role in the management of postmenopausal women with hormone-dependent breast cancer. Previously, AIs have proven effective in postmenopausal women with metastatic breast cancer in whom tamoxifen has failed [8]. Breast cancers in patients treated with AIs as first-line therapy for metastatic disease are likely to eventually become resistant, but may still respond to another type of HT. Therefore, as these AI agents move into the first line, it is essential to establish appropriate second-line therapies. This important clinical question was directly addressed by Long and colleagues [2], using a preclinical model of xenografts with intratumoral aromatase.

Intratumoral aromatase preclinical model

Clinical evidence suggesting that local production of estrogen may contribute to breast tumor growth and, therefore, that intratumoral aromatase is a potential therapeutic target [9] had led Brodie's research group, almost 10 years ago, to develop a valuable preclinical mouse model of intratumoral aromatase [5,10]. Tumors formed by ER-positive MCF-7 human breast cancer cells stably transfected with the human aromatase gene (MCF-7Ca) were grown in ovariectomized nude mice. These tumor cells remain hormone-dependent and, in the presence of aromatase substrate, synthesize sufficient estrogen to stimulate tumor formation and progression [5]. This system, which models the low-estrogen state in postmenopausal women, has proven to successfully predict some HT effectiveness in such women with breast cancer. Thus, previous studies from this model have shown that AIs, and especially letrozole, are more effective at suppressing tumor growth than either tamoxifen or the pure potent anti-estrogen fulvestrant (ICI 182,780; Faslodex) [5,11], and that the combination of AIs with anti-estrogens is no better than treatment with an AI alone. The superiority of AI agents over tamoxifen for the treatment of postmenopausal women with advanced breast cancer [6,12] and also over the combination of an AI plus tamoxifen in the adjuvant setting [7], has indeed been confirmed in several key clinical trials. However, contrary to the preclinical model prediction, two recent randomized phase III trials comparing anastrozole with fulvestrant showed either that fulvestrant is as effective as anastrozole [13] or that fulvestrant is superior to anastrozole [14] for advanced breast cancer in postmenopausal women. Such discrepancies between the preclinical model and results from human trials may be related to some differences in

doses, artifacts of single-cell-line analysis, or intrinsic differences in pharmacokinetics and metabolism of drugs between human and mouse. These discrepancies emphasize some limitations of preclinical models in general, and of the intratumoral aromatase model in particular, and should be taken into consideration when translating these findings into the clinic.

Sequential therapy after first-line treatment with aromatase inhibitor

The establishment of the optimal sequence of endocrine therapies offers significant benefits to women with hormone-sensitive metastatic breast cancer by prolonging the treatment period during which HT can be used [8]. In a recent study, Long and colleagues, using their intratumoral aromatase model, investigated the optimal second-line therapy for patients who fail AI therapy [2]. The authors have taken both *in vitro* and *in vivo* approaches to define hormonal sensitivities of these AI-resistant tumor cells. *In vitro*, aromatase-transfected MCF-7Ca cells were selected by long-term estrogen deprivation to grow in estrogen-depleted medium (UMB-1Ca). Aromatase activity in these cells did not significantly change. Whole-cell ligand-binding assays showed that these selected cells expressed elevated levels of functionally active ER. Basal ER-transcriptional activity was not affected in these cells (as assessed by the level of progesterone receptor), but estrogen stimulation led to a much higher induction of ER activity than in parental cells. Importantly, however, this increased ER activity did not translate to an increased growth response to estrogen, and the cells did not show hypersensitivity to low estrogen levels, contrasting what was previously shown with estrogen-deprived hormone-resistant MCF-7 cells that do not overexpress aromatase [15]. Nevertheless, like the parental MCF-7 cells [15], UMB-1Ca cells remained sensitive to anti-estrogens, both *in vitro* and *in vivo*.

To model the clinical situation of treating postmenopausal breast cancer patients after the failure of AI, Long and colleagues took a second approach and defined the anti-estrogen sensitivity of letrozole-resistant MCF-7Ca xenografts. Resistant xenografts were first selected by their acquired ability to grow in the presence of the letrozole after long-term treatment; the mechanism of resistance has yet to be defined. Resistant tumors were transplanted and reestablished in naive nude mice in the presence of aromatase substrate, and mice were then treated with either anti-estrogens or AIs. Transplant growth was slowed significantly by tamoxifen and even more effectively by the potent anti-estrogen fulvestrant. Surprisingly, while these resistant tumors were refractory to the AIs anastrozole and formestane, they regained significant sensitivity to letrozole. The results therefore suggest that tumors that have failed AI therapy would remain sensitive to second-line therapy with anti-estrogens, and that they may also

respond to rechallenge with AI therapy. Some clinical data have also suggested the possibility that resistance to nonsteroidal AIs would not confer cross-resistance to the steroidal AIs, and vice versa [8]. Whether the increased cellular ER levels in the resistant tumors are responsible for their enhanced sensitivity to anti-estrogen is still an open question. Without doubt, further studies to investigate the underlying mechanisms responsible for letrozole resistance are crucial for a better understanding of how to circumvent or overcome this resistance in the clinic.

The SERM arzoxifene in breast cancer prevention and treatment

Since estrogens have a crucial role in breast cancer initiation, proliferation, and metastasis [16], AIs and parallel methods to eliminate endogenous estrogen may offer a potent strategy for breast cancer treatment and prevention. However, estrogens also play an indispensable role in other organs [17], and therefore SERMs, which possess anti-estrogen activity in the breast but preserve some fundamental estrogen-like functions in other tissues, may offer a more comprehensive and balanced tactic. While tamoxifen was the first widely used breast cancer prevention agent, and is still, along with raloxifene, under active clinical testing for prevention applications, novel SERMs with a more desirable tissue profile are needed. Because of significant advances in understanding the structure and function of ER, several leading and promising compounds are now under clinical development.

As discussed in two recent reports by Suh and colleagues of Sporn's group [3,18], the SERM arzoxifene (Arz) is one of the more promising compounds. It is a new benzothio-phenone derivative similar to raloxifene. Arz acts as a potent anti-estrogen in breast and uterine tissues [19] but possesses estrogen agonist activity to maintain bone density and serum cholesterol. In comparison with tamoxifen, Arz has shown a stronger and more durable antagonist activity in breast cancer cell lines and xenografts [20,21]. Several recent phase II clinical trials also reported high antitumor activity of Arz in advanced breast and endometrial cancers [22]. In their first report regarding Arz, Suh and colleagues showed that Arz is a highly effective agent that is significantly more potent than raloxifene for the prevention of mammary tumors in a rat model [18]. All of the above studies suggest that the SERM profile of Arz is excellent, and that this agent therefore has great promise for breast cancer treatment and prevention.

Arzoxifene in combination with the rexinoid LG 100268 (LG268)

Recent preclinical studies have revealed the potential of combinations of HT with other agents for both prevention and treatment of breast cancer. These agents may act indirectly through the ER, mainly by influencing ER bidirec-

tional crosstalk with other signaling pathways, or may act independently of the ER pathway and affect stromal as well as epithelial cells [23]. The rexinoids, selective ligands for the retinoid X receptors [24], are more effective in tissues that contain ER protein [25] and they exhibit great potential for both chemoprevention and therapy of cancer. Recent studies have also shown that rexinoids can prevent ER-negative breast cancer as well [26].

In a follow-up report on Arz, Suh and colleagues [3] now demonstrate the high efficacy as single agents, and the striking synergy when combined, of Arz and the new rexinoid compound LG268 [24] for *both* treatment and prevention of breast cancer. The authors again used the *N*-nitrosomethylurea (NMU) rat model, an established model for breast cancer development and progression that produces hormone-dependent tumors [27] and has been used successfully to predict the efficacy of chemopreventive hormonal agents. Prevention efficacy against either early or more advanced premalignant lesions was tested by starting the treatment either early or late after the administration of NMU. Prevention of progression of early lesions, which was first demonstrated for the LG268 compound in that paper, was dosage dependent for both drugs. Importantly, when the two drugs were combined, only very low dosages of both were needed to cause meaningful reduction in tumor burden, a significant observation for the clinical setting to minimize toxicity. The high efficacy of each of the drugs and their synergism, though with higher dosages than in the prevention setting, was further achieved in the treatment setting for both early and advanced invasive breast cancer. As a single agent, each drug was capable of slowing or arresting tumor growth, but when the drugs were combined, a dramatic synergism was seen, which resulted in almost complete tumor shrinkage of even very large tumors in as short a time as 3 weeks after the beginning of treatment.

Although some of the inhibitory effects of both Arz and LG268 are very likely due to direct actions on premalignant or malignant mammary epithelial cells, Suh and colleagues hypothesized that part of the synergistic effect may also be mediated by an effect on the stromal cells that control the microenvironment of the tumor. To investigate this hypothesis, they conducted several experiments. Fibroblast cells were treated with Arz and LG268, either alone or in combination, and the effect of their conditioned medium on the growth of lung epithelial cells highly sensitive to transforming growth factor β (TGF- β) [28] was monitored. Growth inhibition of the epithelial cells was induced only by medium derived from fibroblasts treated with the combination of Arz and LG268, and not when the drugs were administered as single agents. This inhibitory effect was most likely related to TGF- β , since a blocking antibody to TGF- β completely reversed the inhibition. Furthermore, by examining induction of the important

angiogenic factor inducible nitric oxide synthase (iNOS) in the fibroblast cells, which is known to be inhibited by TGF- β [29], the authors found that the induction was almost entirely blocked by the combination of Arz and LG268, whereas the compounds had little effect on the expression of iNOS when used alone. Obviously, other mechanistic studies, especially in the context of the NMU-rat model, are needed to investigate further the intriguing hypothesis that stromal TGF- β induction [30] is the conduit by which Arz and LG268 synergistically induce tumor regression, and to reveal other underlying mechanisms that are responsible for this synergistic action on breast cancer prevention and treatment. But regardless of these future studies, the data discussed above, as also concluded by the researchers, strongly suggest that clinical application of the combination of Arz and LG268 should be considered for prevention as well as for treatment in both the adjuvant and advanced breast cancer settings.

Competing interests

None declared.

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