Targeting androgen receptor in BQ323636.1 overexpressing oestrogen receptor-positive breast cancer to overcome aromatase inhibitor resistance: abridged secondary publication

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KEY MESSAGES

1. BQ323636.1 overexpression enhances the activity of androgen receptor (AR) signalling and reverses the tumour suppressive effect of aromatase inhibitors (AI).
2. High nuclear BQ323636.1 (BQ) and AR expression is associated with AI resistance and poorer survival in post-menopausal oestrogen receptor-positive (ER+) breast cancer patients.
3. Co-treatment with AR inhibitor, bicalutamide can recover the therapeutic effect of AI in ER+/BQ+/AR+ breast cancer.

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Introduction

Breast cancer can be classified into hormonal receptor-positive, HER2 overexpressed, and triple-negative breast cancer subtypes, and treatment for each subtype differs entirely. Most oestrogen receptor-positive (ER+) tumours initially depend on ER-activation by the steroid hormone oestrogen. Oestrogen-induced ER activation promotes proliferation and survival of breast tissue through transcription of pro-survival genes (genomic regulation) and activation of cellular signalling (non-genomic regulation).[1] Activated ER can promote cell proliferation, leading to cancer development. Oestrogen suppression remains the mainstay of ER+ breast cancer treatment.

Adjuvant endocrine therapy is recommended for patients with ER+ breast cancer to prevent metastasis and local recurrence. The aromatase inhibitors (AI) such as anastrozole, letrozole, and exemestane are options for post-menopausal patients. We previously identified a novel splice variant of NCOR2 and BQ323636.1 (BQ), which present in most primary breast cancers and associated with tamoxifen resistance.[1] NCOR2 is a nuclear receptor co-repressor that suppresses transcription through interaction with transcription factors. ER-α and androgen receptor (AR) are targets of NCOR2.[2] Although NCOR2 can repress the activities of ER and AR, BQ overexpression abolishes the repressive role of NCOR2 by forming a BQ-NCOR2 functionless dimer, leading to ligand-independent activation of ER.[3] High nuclear BQ expression is associated with tamoxifen resistance.[3] We proposed that BQ might use a similar mechanism to enhance the activity of AR in breast tumours. Hijacking AR signalling is the driving factor of prostate cancer. AI inhibits the conversion of androgen to oestrogen to repress ER signalling. Thus, AI treatment can lead to more androgen being available for enhanced AR activity. This increased availability of androgen together with enhanced AR activity in BQ-overexpressing cells might lead to over-activation of AR signalling, which could have an oncogenic effect. We hypothesise that AR over-activation in BQ-overexpressing cells can interfere with the tumour suppressive effect of AI. This study illustrates that supressing AR can recover the therapeutic value of AI in ER+/AR+/BQ+ breast cancer.

Inhibition of AR signalling to recover tumour suppressive effect of AI in ER+/BQ+/AR+ breast cancer in vitro and in vivo

After the maximum non-lethal dosage of the AR antagonist—bicalutamide (BIC)—was determined, cell viability assay showed that co-treatment with AI and BIC in BQ-overexpressing cells (MCF-7-BQ and ZR-75-BQ) resulted in reversal of the effect of AI treatment alone. Moreover, luciferase reporter assay showed that the addition of BIC abolished the effect of AI on ARE-activity. Cell cycle analysis via flow cytometry suggested that the addition of BIC could recover the cell cycle inhibition effect of AI. Western blot analysis confirmed this notion by detection of CDK2, CDK4, and CCNE1. These

results demonstrated that applying BIC on BQ-overexpressing cells could recover the tumour suppressive effect of AI.

To evaluate the effect of AR inhibition on recovering AI's efficacy in BQ-overexpressing tumours, ZR-75 and ZR-75-BQ cells transfected with CYP19A1 were implanted onto the mammary fat pad of ovariectomised nude mice. Treatment of 10 mg/kg of Arimidex, clinically used AI for menopausal ER+ breast cancer patients, could significantly suppress tumour development in ZR-75 xenograft. Similarly, AI alone enhanced tumour development in BQ overexpressed xenograft ZR-75-BQ; this effect was reversed by co-treatment with AR antagonist Casodex, the clinical equivalent of bicalutamide.

Clinical significance of BQ and AR in post-menopause ER+ breast cancer

In patients who had been treated with AI for at least 5 years, the expression of BQ and AR of 267 primary breast carcinomas in tissue microarray was examined by immunohistochemistry. High expression of BQ and AR was associated with AI resistance.

Kaplan-Meier survival analysis showed that patients with high BQ expression had poorer overall survival, disease-specific survival, and disease-free survival. Therefore, high nuclear BQ could be an indicator of poorer prognosis in ER+ post-menopausal patients with breast cancer who received AI adjuvant therapy. When combined expression of both BQ and AR was used, patients with both high BQ and AR expression also showed poorer overall survival, disease-specific survival, and disease-free survival, with greater discrimination in the combined analysis with survival curves further apart.

Discussion

Endocrine therapies such as selective ER modulators, selective ER down regulators, and AIs are used for adjuvant treatment of ER+ breast cancer. AI depletes systemic oestrogen in post-menopausal patients by blocking the conversion of androgens to oestrogens. AI therapy results in a more significant reduction in the risk of recurrence than 5-year tamoxifen therapy such that most post-menopausal women should consider AI treatment either as initial therapy or after 2 to 3 years of tamoxifen therapy. However, the occurrence of resistance is an obstacle.

Our study found that BQ overexpression in primary cancer could change the therapeutic effect of AI into a tumour-promoting agent. AI suppresses the activity of aromatase to convert androgen to oestrogen and thus suppress ER-signalling in ER+ breast cancer. However, the unconverted androgen might activate AR-signalling in the presence of AR. Luciferase reporter assay confirmed that BQ-overexpression could amplify AR-signalling intensity when AI was used. BQ is a splice variant of NCOR2.[1] NCOR2, being a co-repressor, forms a repressor complex to suppress transcription mediated by different transcription factors such as ER and AR.[2] BQ overexpression reduces the suppression of ER-signalling, resulting in tamoxifen resistance.[3] Likewise, it could also reduce the suppression of AR-signalling. AI treatment aims at eliminating the conversion of androgen. As a result, in terms of BQ overexpression, AI would work together to enhance the activity of AR signalling. AR expression is detected in about 60% to 90% of ER+ breast cancer. Thus, enhanced AR-signalling mediated by AI in BQ overexpressing breast cancer becomes a tumour driving factor. Suppression of AR signalling should reverse such an effect.

In patients with high BQ and AR expression, AI treatment was ineffective and even tumour-promoting. The use of an AR antagonist could reverse this effect. These findings are confirmed in primary breast cancer mouse models. High expressions of BQ and AR in ER+ patients treated with AI were associated with AI resistance and poorer survival outcomes. Therefore, we recommend examining the nuclear expression of AR and BQ in the primary breast cancer by immunohistochemistry.

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Disclosure

The results of this research have been previously published in:

1. You CP, Leung MH, Tsang WC, Khoo US, Tsoi H. Androgen receptor as an emerging feasible biomarker for breast cancer. Biomolecules 2022;12:72.
2. Tsoi H, Shi L, Leung MH, et al. Overexpression of BQ323636.1 modulated AR/IL-8/CXCR1 axis to confer tamoxifen resistance in ER-positive breast cancer. Life (Basel) 2022;12:93.

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