Characterising an aromatase inhibitor-resistant breast cancer cell line

Harris E[1],
Varešlija D[2],
O'Hara J[2],
Hill AD[2],
Young LS[2]
[1]RCSI medical student
[2]Endocrine Oncology Research, Department of Surgery, RCSI

Background

Aromatase inhibitors (AIs) are a novel adjuvant endocrine treatment for oestrogen receptor (ER)-positive breast cancer in postmenopausal women. They inhibit the conversion of androgens to oestrogens. In the clinic, AIs have been shown to be superior to tamoxifen (a selective ER modulator), with improved tolerability and increased disease-free survival.[1,2] However, prolonged use of AIs can lead to acquired resistance characterised by aberrant ER signalling and crosstalk with growth factor pathways.[2] An AI-resistant breast cancer cell line, Let-R, is currently being investigated and demonstrates differential oestrogen regulation of target genes. In resistance, classical genes such as pS2 become oestrogen-independent. However, cyclinD1 remains oestrogen-regulated and appears to be modulated through oestrogen signalling to c-jun N-terminal kinase (JNK) in addition to the ER. This study aims to characterise the Let-R cell line created in the lab and to optimise an ER-α knockdown in Let-R cells.

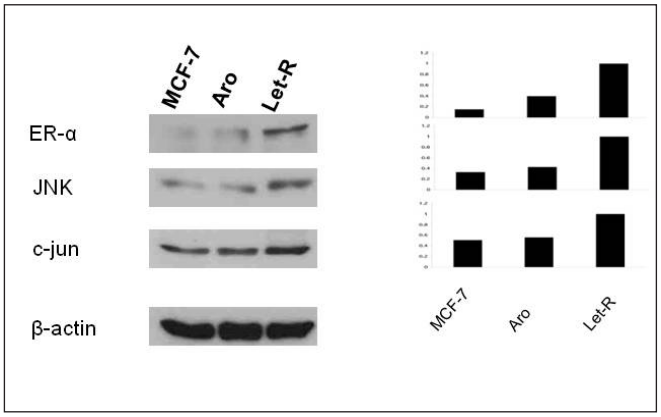

FIGURE 1: Western blot demonstrating relative ER-α, JNK and c-jun expression in endocrine-sensitive MCF-7, AI-sensitive Aro and AI-resistant Let-R cell lines.

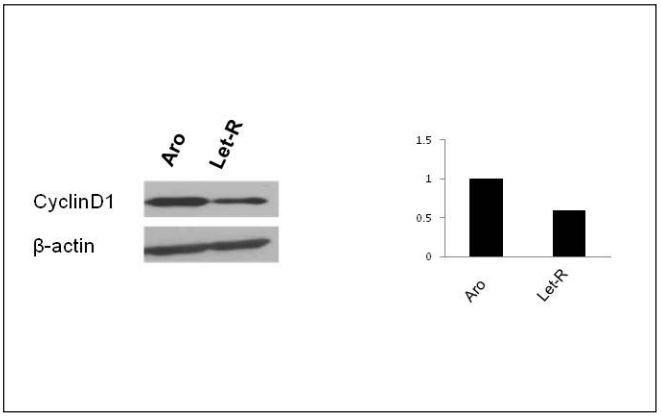

FIGURE 2: Western blot demonstrating relative cyclinD1 expression in AI-sensitive Aro and AI-resistant Let-R cell lines.

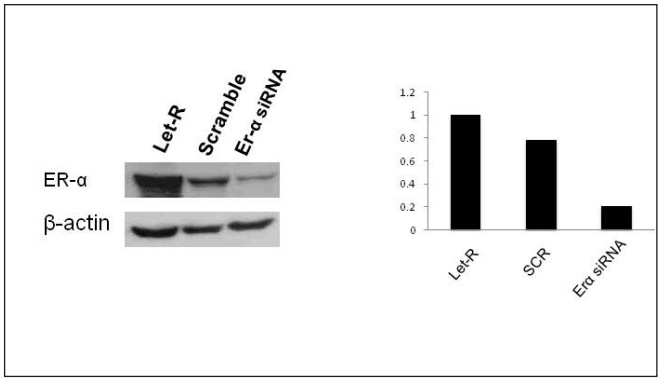

FIGURE 3: Western blot demonstrating ER-α knockdown in Let-R cells using RNA interference.

Methods

Endocrine-sensitive parental MCF-7, AI-sensitive MCF-7-Aro and MCF-7-AroR-Let (Let-R) breast cancer cell lines were used to investigate protein expression levels of ER-α, cyclinD1, JNK and c-jun using western blotting. Cells were transfected with 100pmol of ER-α small interfering RNA for 48 hours to optimise the knockdown in Let-R cells.

Results

Let-R cells have higher levels of ER-α, JNK and c-jun compared to MCF-7-Aro cells (**Figure 1**). CyclinD1 basal levels are elevated in MCF-7-Aro cells compared to Let-R cells (**Figure 2**). An effective ER-α knockdown in Let-R cells was optimised (**Figure 3**).

Conclusion

The significant ER-α knockdown achieved in the Let-R cells enables further examination of knockdown effects on protein levels and mRNA expression.

Elevated expression of ER-α in the Let-R cells observed is consistent with the association between AI resistance and ER hypersensitivity.[3] In resistant cells, the decrease in basal levels of cyclinD1 may be partly responsible for the lack of regulation of classical genes like pS2, previously detected in the laboratory.[4] The elevated levels of JNK and c-jun observed in Let-R cells support the theory of crosstalk between the ER and growth factor pathways in resistance.[2] It would be of interest to accompany AI treatments with growth factor inhibitors to further our understanding of endocrine resistance. Understanding such resistance mechanisms will help to create novel biomarkers and therapies to predict and overcome AI resistance.

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References

1. Goss PE *et al.* Randomised trial of letrozole following tamoxifen as extended adjuvant therapy in receptor-positive breast cancer: updated findings from NCIC CTG MA.17. J Natl Cancer Inst. 2005;97(17):1262-71.
2. Jordan VC, Brodie AM. Development and evolution of therapies targeted to the oestrogen receptor for the treatment and prevention of breast cancer. Steroids. 2007;72(1):7-25.
3. Santen RJ *et al.* Adaptation to oestradiol deprivation causes up-regulation of growth factor pathways and hypersensitivity to oestradiol in breast cancer cells. Adv Exp Med Biol. 2008;630:19-34.
4. Loden M *et al.* The cyclinD1 high and cyclin E high subgroups of breast cancer: separate pathways in tumorogenesis based on pattern of genetic aberrations and inactivation of the pRb node. Oncogene. 2002;21(30):4680-90.