Assessing ER Activity in Hormonal Therapy Drug Resistant Cells

Omotayo Ajibolade Supervisor: Dr. Mark Wade & Dr. Luke Gaughan, Northern Institute of Cancer Research.

Introduction

ER is a major driver of growth and development in the majority of breast cancers. Hormonal therapies which target the ER, such as the drugs Tamoxifen and Fulvesterant, are available for breast cancer treatment but many cancers become resistant to these therapies. Understanding the mechanism of resistance is therefore crucial in treating these cancers.

This Project is aimed at assessing ER activity in drug resistant cell lines developed from ER positive MCF-7 cells, to investigate the role of ER in growth of resistant disease.

Methods

Cell Culture and harvest: 7 months before my placement, MCF-7 cell lines were grown in different concentrations of Tamoxifen and Fulvestrant to make them resistant to the drugs. Cells from all resistant cell lines and Parental MCF-7 cells were grown in estrogen starved conditions and then treated with or w/out drugs and estrogen and harvested for protein and RNA analysis after three days.

Western Blotting Protein extracted from parental MCF-7, Tamoxifen and Fulvestrant treated parental MCF-7 and drug resistant MCF-7 cells were probed for α tubulin to determine the absolute amounts of the proteins present before probing for ER.

QPCR: RNA was extracted from the different cell lines and cDNA was generated by reverse transcription. Quantitative-PCR (QPCR) was carried out on cDNA to assess the expression of several ER-target genes, including *pS2*, *GREB1* and *c-MYC* in the resistant cell lines compared to parental cells.

Results

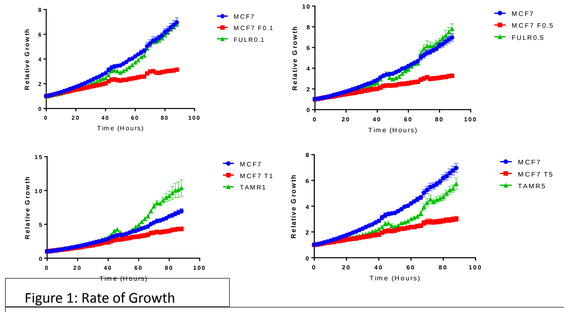

Figure 1: Rate of Growth

Previous work done by my supervisor assessed the rate of growth of parental MCF-7 cells, MCF-7 cells treated with Tamoxifen and Fulvestrant and drug resistant cells. Figure1 indicates that although the rate of growth of MCF-7 cells reduces when treated with 0.1μM & 0.5μM Fulvestrant and 1μM & 5μM Tamoxifen, the rate of cell growth of resistant cell lines (TAMR1, TAMR5,FULR0.1 & FULR0.5) does not reduce. This indicates that resistance has occurred after growing cells in the drugs over a long period of time.

Western Blotting

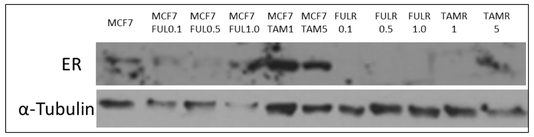

Figure 2: Western blot showing levels of ER and α-tubulin in Parental MCF-7 cells, Tamoxifen and Fulvestrant treated MCF-7 cells and tamoxifen & Fulvestrant resistant cells

Figure 2

Lanes 2-4 of the western blot show an absence of ER in MCF7 cells treated with Fulvestrant in comparison to lane 1 parental MCF7 cells. This indicates that Fulvestrant reduces ER levels, as expected. Lane 5&6 on the other hand shows the presence of ER after being treated with Tamoxifen, these results indicate that tamoxifen does not reduce ER levels but instead competes with estrogen for its active site.

In Lanes 6-10 it was observed that in Fulvestrant- and Tamoxifen-resistant MCF-7 cell lines, ER levels were almost undetectable, suggesting that the cells had developed an ability to proliferate without the need of ER.

QPCR

Considering ER does not appear to be expressed in resistant cell lines I performed quantitative PCR (QPCR) to assess gene expression of the ER and ER-target genes, including *pS2*, *GREB1* and *c-MYC*.

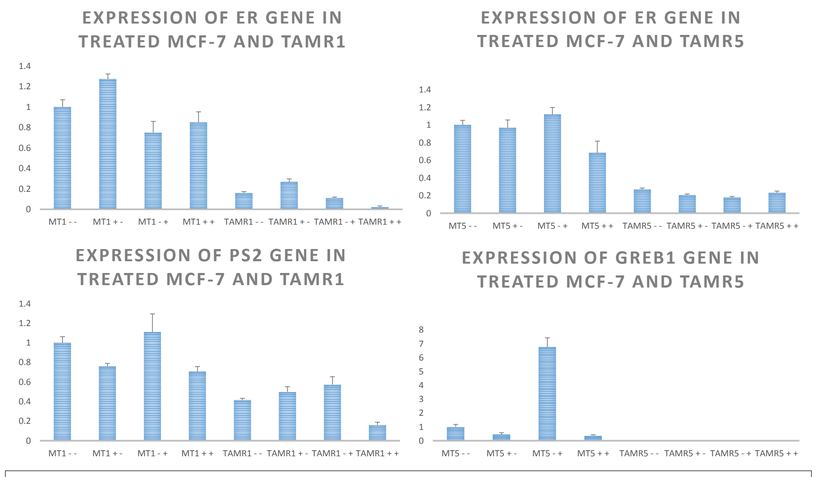

Figure 3: Graphs showing the expression of ER, *pS2 and GREB1*genes in MCF-7 or TAMR cell lines when treated or not treated in 1μM or 5μM Tamoxifen(indicated with first +/-) in the presence or absence of estrogen (indicated with second +/-)

Key:

Cell Lines

TAMR1 &TAMR5 are resistant MCF-7 cell lines treated in 1μM and 5μM Tamoxifen.

FULR0.1, FULR0.5 and FULR1 are resistant MCF-7 cell lines treated in 0.1μM, 0.5μM and 1μM Fulvestrant.

Graphs:

Eg:MT1++: MCF7 cells treated in 1μM Tamoxifen with oestrogen present, MF0.1--; MCF7 cells not treated with 0.1μM Fulvestrant in the Absence of Estrogen.

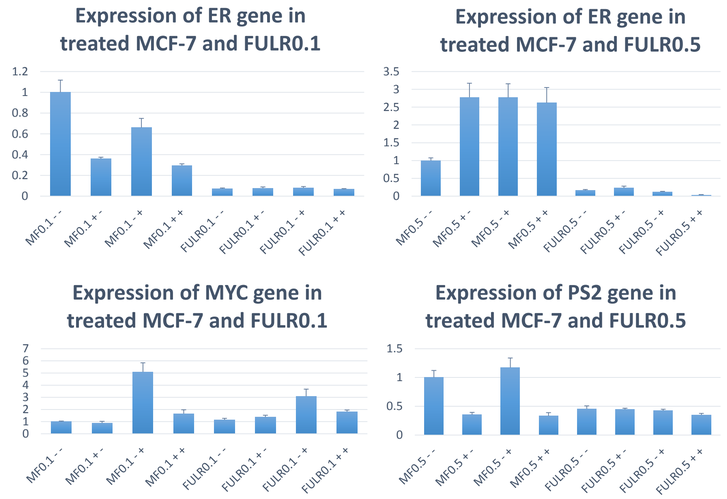

Figure 4: Graphs showing the expression of ER, *pS2 and MYC* genes in MCF-7 or TAMR cell lines when treated or not treated in 0.1μM or 0.5μM Fulvestrant (indicated with first +/-) in the presence or absence of estrogen (indicated with second +/-)

As expected Figure 3 and 4 support the western results; ER genes are expressed in Tamoxifen and Fulvestrant treated MCF7 cell lines and the genes are down regulated in resistant cell lines proving that cells are growing without functional ER. Also as expected parental MCF-7 cells in all three ER target genes demonstrated robust up-regulation in response to estrogen. In contrast, pS2, GREB1 and c-MYC were not up-regulated by estrogen treatment in the Tamoxifen- and Fulvestrant-resistant cell lines. These preliminary data suggests that the Tamoxifen- and Fulvestrant-resistant MCF-7 derivatives have developed a way to grow without a functional ER.

Conclusion

This work gives an insight into the potential mechanisms of hormonal therapy-resistance in breast cancer. The results indicated that ER levels in these resistant cell lines are reduced and hence no longer respond to estrogenic stimulation. A key extrapolation to these preliminary experiments is to define the main drivers of cell proliferation in these derivatives and exploit them for potential future therapies.

Acknowledgment

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Omotayo Ajibolade, Physiological sciences, o.h.ajibolade@newcastle.ac.uk ,130064910.