Validation of the histone demethylase enzyme KDM3A as an estrogen receptor regulator in the tamoxifen-resistant breast cancer cell line MMU2

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Background – Breast cancer is the most common malignancy in women, with 1 in 9 expected to develop the disease in the UK. Many cases express the estrogen receptor (ER), a transcription factor that controls proliferation and transformation of breast cells. Conventional hormonal therapies, such as Tamoxifen, act to inactivate the ER and are initially successful. However, most malignancies develop resistance to Tamoxifen, and with limited second-line therapy options, many are fatal. There is an urgent need, therefore, to identify new targets for advanced disease treatment.

Introduction

– KDM3A is a histone demethylase enzyme and has been identified as a positive regulator of the ER. Data from the laboratory has shown KDM3A removes repressive histone methylation marks from ER-target genes to enhance their transcription in several breast cancer cell lines. Importantly, the role of KDM3A in Tamoxifen-resistant disease was unknown. The aim of this study, therefore, was to investigate the effect of KDM3A depletion on cell growth and expression of ER-regulated genes in Tamoxifen-resistant MMU2 breast cancer cells and compare to Tamoxifen-sensitive MCF-7 cells.

Methods

- The knockdown of KDM3A was achieved by transfection of two siRNA oligonucleotides: siKDM3A B and siKDM3A C and compared to a control scrambled siRNA (Scr). ER was depleted using siRNA ER. All siRNAs were used at a final concentration of 20 nM
- Western blots were carried out using KDM3A, ER and alpha-tubulin (control) antibodies.
- qPCR was performed using primers for ER target genes (*pS2*, and *CCND1*) to asses gene expression.
- Cell growth was measured using the Incucyte zoom machine, in which cells were grown and confluence measured.

Results

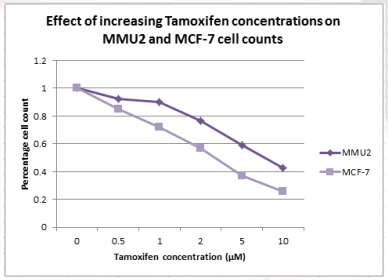

Figure 1. Graph showing how increasing doses of Tamoxifen reduces MCF-7 numbers rapidly, but at a slower decline with the MMU2s.

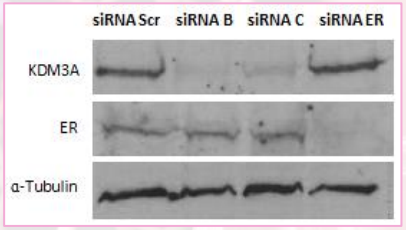

Figure 2. MMU2 cells showing KDM3A knockdown by siKDM3A B and C oligonucleotides. ER western blot shows no reduction in response to KDM3A knockdown. α-tubulin was used as a loading control

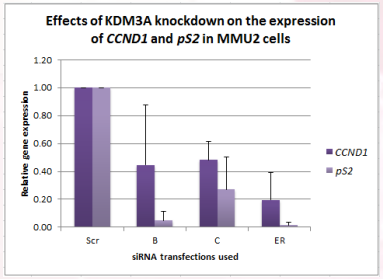

Figure 3. qPCR results for *CCND1* and *pS2* from two experiments showing a decrease in expression with KDM3A knockdown which is similar to ER knockdown.

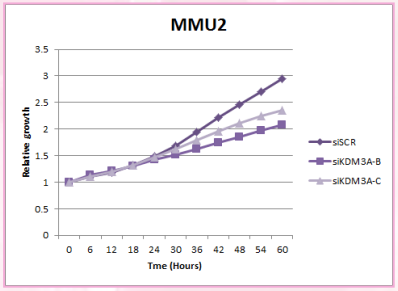

Figure 4. Incucyte data showing reduced growth of cells depleted of KDM3A compared to control.

Discussion

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1. As expected, MMU2 cells demonstrated less sensitivity to Tamoxifen than MCF-7 cells.
2. KDM3A knockdown decreases expression of the ER-target genes *pS2* and *CCND1* and reduces cell growth indicating a role for KDM3A in regulating ER and cellular phenotype of the MMU2 cells.
3. The implications of this study allow future investigation into developing late stage drugs for Tamoxifen-resistant cases of the disease.

References

1. Johnson N, Speirs V, Curtin N.J, Hall A.G (2008) *Breast Cancer Res Treat* **111**, 55-63
2. Johnston SR, Saccani-Jotti G, Smith IE, et al. (1995) *Cancer Research* **55**, 3331-8