Investigating the Role of GATA2 in the DNA Damage Response in Prostate Cancer

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Objectives

- Reduce the expression of the GATA2 gene in the advanced prostate cancer (PC) cell line CWR22Rv1
- Observe the effect of reducing GATA2 gene activity on:
  - The expression of several DNA damage repair genes required for tumour survival
  - The persistence of DNA damage following the treatment of PC cells with ionising radiation

Introduction

PC is the most common cancer in men, affecting 1 in 8 males during their lifetime.[1]

The primary target of PC therapy is the androgen receptor (AR) as it is established that this protein drives cancer progression. Unfortunately, most patients become resistant to this treatment, through mechanisms that enable persistence of AR activity; and the survival benefit of subsequent radiation- and chemo-therapy is minimal. It is therefore important that new targets for disease therapy are identified to help improve treatment of advanced stage PC patients.[2]

Pilot work from my host laboratory has identified that a protein called GATA2 promotes activity of the AR and may be important for enabling the cell to counteract the DNA-damaging and cancer cell-killing effects of both radiation- and chemo-therapies.

We hypothesise that by reducing GATA2 levels in models of advanced PC, cells will have compromised AR activity and will be less proficient at being able to repair DNA upon radiation- and chemo-therapy.

Methods

RNA TRANSFECTION

In order to reduce GATA2 expression, siRNA complimentary to GATA2 mRNA was transfected into the CRW22Rv1 PC cells for 48 hours. This process prevents production of the GATA2 protein in the cell by destabilising the target mRNA. In addition, cells were also transfected with a control siRNA that mimics the process of transfection, but does not impact directly on GATA2 protein levels. Cells were cultured in the presence or absence of Enzalutamide (+/-Enz), an inhibitor of the full-length AR for the final 24 hours of the experiment.

WESTERN BLOT

Cells were lysed and proteins separated according to their molecular weight using polyacrylamide gel electrophoresis, prior to electro-transfer of proteins onto a nitrocellulose membrane. Membranes were subsequently probed with a GATA2-specific antibody (Santa Cruz Biotechnology) followed by an appropriate secondary antibody to enable detection of GATA2 by autoradiography. Comparison between a control and GATA2 knock down (KD) sample provides evidence of whether the RNA transfection procedure has been successful.

RNA EXTRACTION

RNA was isolated from siRNA-transfected CWR22Rv1 cells using Trizol (Life Sciences) and reverse transcribed to produce cDNA using MMLV reverse transcriptase (Promega). cDNA was subsequently incorporated into quantitative PCR using primers specific to several key DNA damage-associated genes. This enabled comparison of the activity of these genes to a control sample in which the GATA2 protein levels were not depleted.

IONISING RADIATION

CWR22Rv1 cells transfected with either GATA2 or control siRNAs were subjected to treatment with ionising radiation to cause damage to DNA. A fluorescent tag specific to a biological marker for DNA damage (γH2AX foci) was added to the cells and the samples visualised using a fluorescence microscope. This enabled detection of variation between the control and GATA2-depleted cells in the amount of DNA damage remaining following exposure to radiation.

Results & Discussion

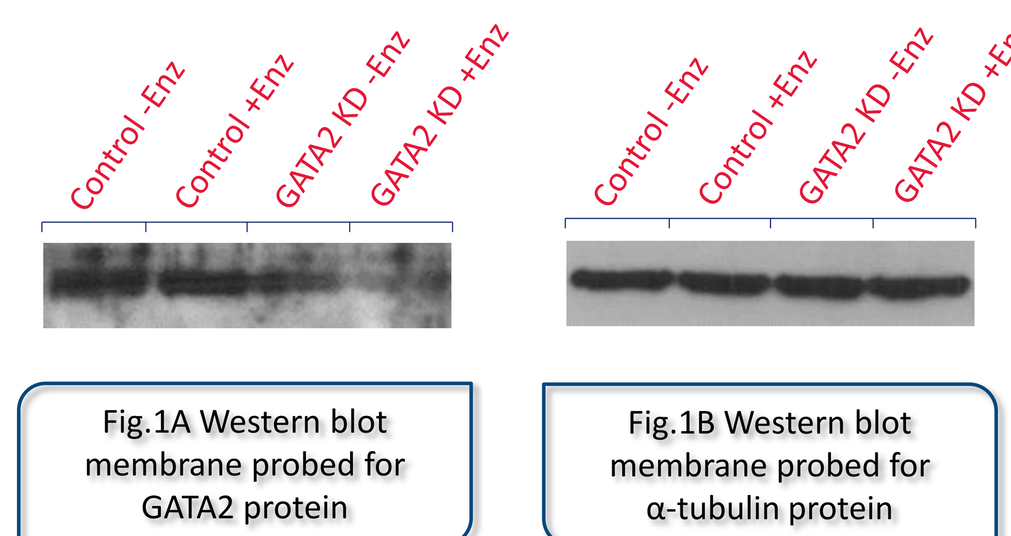

Fig.1A Western blot membrane probed for GATA2 protein

Fig.1B Western blot membrane probed for α-tubulin protein

In Figure 1A, a reduction in the abundance of the GATA2 protein is observed in the GATA2 KD sample compared to the control. As expected, no variation was observed between cells grown with or without Enzalutamide as they were cultured in a steroid-free medium and hence the androgen receptor is inactive. A control experiment detecting the presence of α-tubulin, a protein found in all cells, can be seen in Figure 1B. The consistent levels across the 4 samples show that any differences seen in Fig.1A are not due to variation in total protein quantity utilised in the experiment.

Figure 2 shows the expression of 5 DNA damage response genes, determined using quantitative PCR. FEN1, RAD54B, RAD51, SFN1 and UBE2N all displayed a reduction in levels in the cDNA sample from cells with GATA2 depletion compared to the control cells. This suggests that reducing GATA2 activity impairs the ability of the cell to repair DNA damage and may prevent proliferation of cancerous cells.

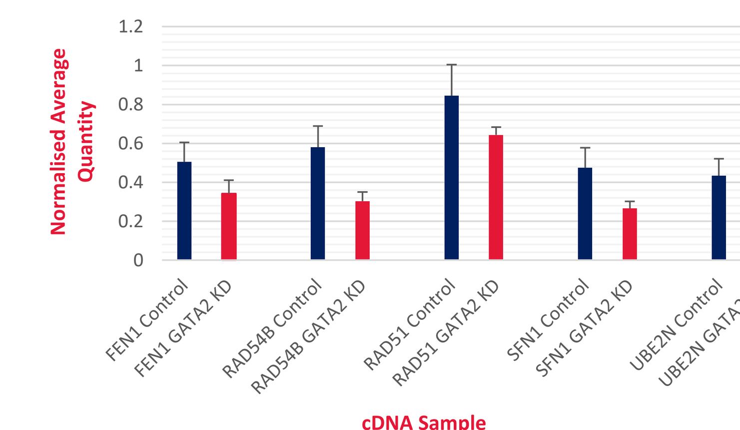

Fig.2 Quantitative PCR data showing DNA damage gene expression in control and GATA2 KD samples. Error bars denote standard deviation values

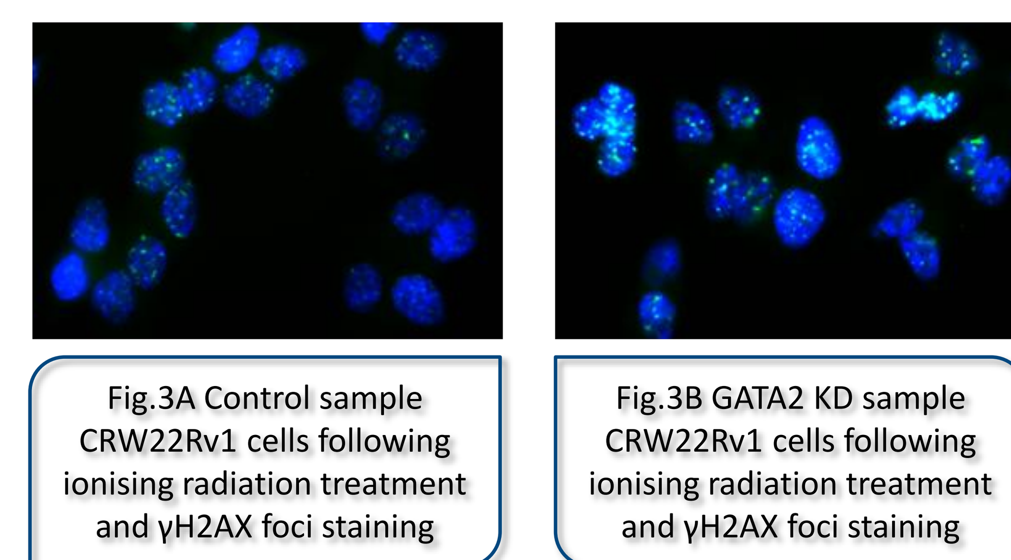

Fig.3A Control sample CRW22Rv1 cells following ionising radiation treatment and γH2AX foci staining

Fig.3B GATA2 KD sample CRW22Rv1 cells following ionising radiation treatment and γH2AX foci staining

Figures 3A and B show immunofluorescence images of PC cells that have been exposed to ionising radiation and stained to allow visualisation of sites of DNA damage (green) within the nucleus (blue). Though more markers of damage (γH2AX foci) appear to be visible in the GATA2 knock down sample, this proved difficult to quantify and larger sample areas should be considered in future to ensure that the results are directly representative of the effects of reducing GATA2 expression in cells. If proven to reduce the ability of the cell to repair DNA damage, inhibiting GATA2 would prevent the cell from dividing and producing a larger malignancy.

Conclusions

- This work found that GATA2 gene knock down caused a decrease in the expression of several DNA damage repair genes and appeared to reduce the cell's ability to respond to DNA damage caused by ionising radiation
- Further work would focus on confirming the reliability of these results by repeating the experiments to allow the identification of a definitive role for GATA2 in the DNA damage response
- If GATA2 inhibition is proved to have beneficial effects on limiting the DNA damage response, thereby preventing cancerous cells from replicating, there is potential for the development of a PC treatment using GATA2 inhibitors

References

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2. He B, Lanz RB, Fiskus W et al. GATA2 facilitates steroid receptor coactivator recruitment to the androgen receptor complex. Proc Natl Acad Sci U S A. 2014 Dec 23;111(51):18261-6.

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