PSMA4 and PSMA6 of the 20S Proteasome and their roles in Androgen Receptor signalling in Prostate Cancer

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Introduction

Background

In the UK 2014, prostate cancer was found to be the second most common cancer overall, with the disease being the most common cancer in males. Fortunately, prostate cancer is very treatable, with an 84% survival rate for 10 or more years between 2010-2011 in England and Wales.

However, individual patients can fail to respond to treatments, including: androgen depletion therapy, antiandrogens (such as Enzalutamide (ENZ)) or orchidectomy which can result in castration-resistant prostate cancer (CRPCa). This is due to the presence of a shortened form of the androgen receptor (AR), termed androgen receptor-variants (AR-Vs). Whereby, the ligand-binding domain of the AR mRNA transcript is truncated. Thus, AR signalling is constitutively active, leading to aberrant cellular activity, despite lack of androgen. The consequence of which causes prostate tumour growth and survival.

The 20S Proteasome

The 26S Proteasome plays a fundamental role in the recycling of cellular components, via the ubiquitin pathway. Recently, it has been discovered that the Proteasome is also responsible for the regulation of AR and AR-Vs to androgen response elements (AREs), such as prostate serum antigen (PSA). Therefore, the 26S Proteasome can be identified as a novel target in the prevention of CRPCa.

But, the initial formation of the Proteasome assumes a 20S complex. Preliminary stages of Proteasome formation involves the assembly of the alpha-ring, whereby PSMA4 and PSMA6 are recruited. This is shown in *Figure 1.*

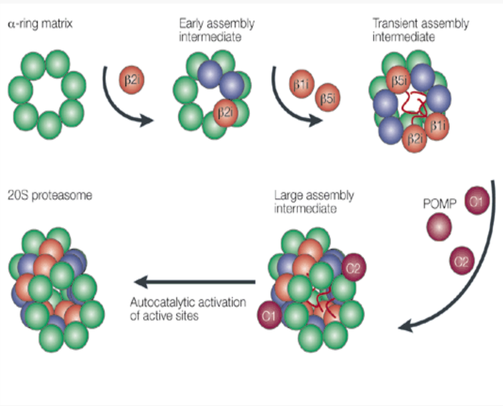


Figure 1 – illustrating alpha-ring assembly as the first step in Proteasome synthesis

Hypothesis, Aims and Objectives

Hypothesis:

Gene knockdown of PSMA4 and PSMA6 decreases AR signalling and subsequently reduces the expression of AREs in CW22Rv1 cells.

Aims and Objectives:

- To investigate the functions of PSMA4 and PSMA6 in AR signalling
- To examine PSMA4 and PSMA6 as potential therapeutic targets in CRPCa
- To compare the individual effectiveness of PSMA4 and PSMA6 knockdowns in inhibiting the recruitment of AR-Vs to AREs

Materials and Methods

Gene knockdown by siRNA transfection:

- CWR22Rv1 cells were reverse transfected with 25nM Scramble (Scr), PSMA4 and PSMA6 small interfering RNA (siRNA) using Lipofectamine® RNAiMax (Invitrogen) in steroid-depleted media.

Treatment with ENZ:

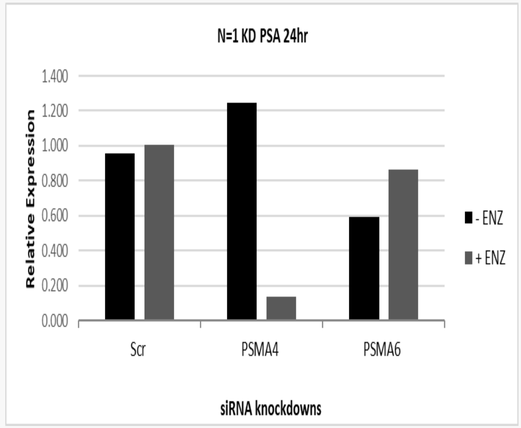
- Appropriate CWR22v1 cells were treated with ENZ (1μM) 24 hours after transfection and incubated another 24 hours.

Protein analysis:

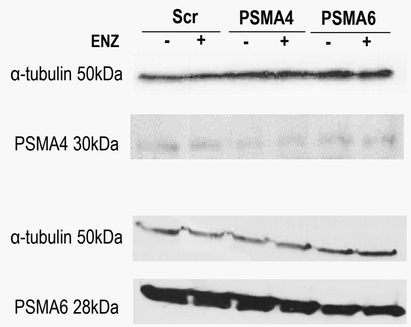
- CWR22v1 cells were lysed for western blot analysis and RNA was extracted for gene expression (QPCR). analysis via quantitative polymerase chain reaction

Results and Conclusions

1. Knockdown of proteasome subunits PSMA4 and PSMA6 compromises transcriptional activity of PSA



2. Demonstrating success of siRNA knockdowns of PSMA4 and PSMA6 via Western Blot Analysis



Conclusions

- Transcriptional activity of PSA is (partially) reduced with knockdown of both PSMA4 and PSMA6.
- Fully intact Proteasome is required for efficient transcription of AREs, such as PSA.

References

- Kloetzel PM (2001) "Antigen processing by the proteasome". Nature Reviews Molecular Cell Biology 2, 179-188. doi:10.1038/35056572
- Lendval N et al. (2012) Phase II Study of Infusional Carfilzomib in Patients with Relapsed or Refractory Multiple Myeloma.".

Discussion

PSMA4 and PSMA6 knockdown proved to be successful due to the decreased relative expression of PSA in all but one of the conditions. Western Blot analysis also reinforces the successive knockdown of the proteasome subunits.

Conversely, PCR analysis for the PSMA4 KD –ENZ group shows a higher relative expression of PSA than the Scr (control) group. This anomaly can be explained by the fact that only one reading of PSA expression was taken. To improve on this, I would take multiple readings for the relative expression of PSA, allowing the calculation of an accurate average, thus, removing any anomalous data. Consequently, a conclusion regarding the individual effectiveness of either a PSMA4 or PSMA6 knockdown on reducing ARE transcriptional activity could not be made.

Moreover, the absence or presence of Enzalutamide seemed to show no effect on the transcriptional activity of PSA. But, we would expect to see a more consistent decrease in the relative expression of PSA, due to Enzalutamide being an antiandrogen. Hence, carrying out more repeats would provide reliable and valid data.

Also, initial knockdown of PSMA4 and PSMA6 subunits were unsuccessful using 7.5μL RNAiMax and 4μL of Scr/PSMA4/PSMA6 oligonucleotide. Western Blot analysis displayed identical band size along the Scr, PSMA4 and PSMA6 lanes. After several failed attempts at demonstrating PSMA4 and PSMA6 knockdown effectiveness, it was decided to alter the experimental parameters. This could have been due to the cell line being older, a consequence of which being that the cells were less prone to transfection. Therefore, we increased the volume of both the Scr/PSMA4/PSMA6 oligonucleotide and RNAiMax transfecting reagent two-fold, which was subsequently used to transfect the CWR22Rv1 cells. This resulted in the successful knockdown of the proteasome subunits, as shown by the Western Blot analysis.

Furthermore, the PSMA4 antibody was shown to be very temperamental. The Western Blot representing PSMA4 knockdown demonstrates this, which explains why the bands are not as prominent when compared to the other bands.

A possible implication of this project could be to investigate a proteasome inhibitor, such as Carfilzomib into the effectiveness of reducing AR signalling. As the knockdown of Proteasome subunits has shown to be effective in reducing AR-medicated signalling. So, proteasome inhibitors may provide potential treatment into CRPCa.

Carfilzomib has already shown to be effective in treating patients with multiple myeloma in clinical trials. The mode of action of the drug is to bind to and inhibit the 20S Proteasome, causing the unwanted build up of polyubiquitinated proteins. The result of which can lead to cellular arrest and apoptosis.