Utilising red and green fluorescence in the detection of alternative splicing in prostate cancer

Development of a high throughput assay to detect compounds regulating the formation of AR splice variants

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BACKGROUND

- Prostate cancer is the most common cancer in men
- In the UK, 1 in 8 men will get prostate cancer
- Age, family history and ethnicity are risk factors for prostate cancer

INTRODUCTION

- Alternative splicing increases diversity in proteins from genes
- In prostate cancer (PCa) there are shortened versions of the full length androgen receptor (AR) (shown in *Figure 1*), called androgen receptor variants (AR-Vs)
- AR-Vs are constitutively active and lack the ligand binding domain at the C terminus

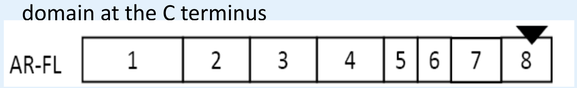


Figure 1: Full length androgen receptor showing exons 1-8 and ligand activation on exon 8

- AR-Vs are often resistant to current therapies (often hormonal therapies) and lead to a more aggressive form of cancer known as castrate-resistant prostate cancer (CRPC)
- At the moment it is unclear what compounds and proteins are involved in the formation of AR-Vs
- If this was understood it could provide new therapeutic targets

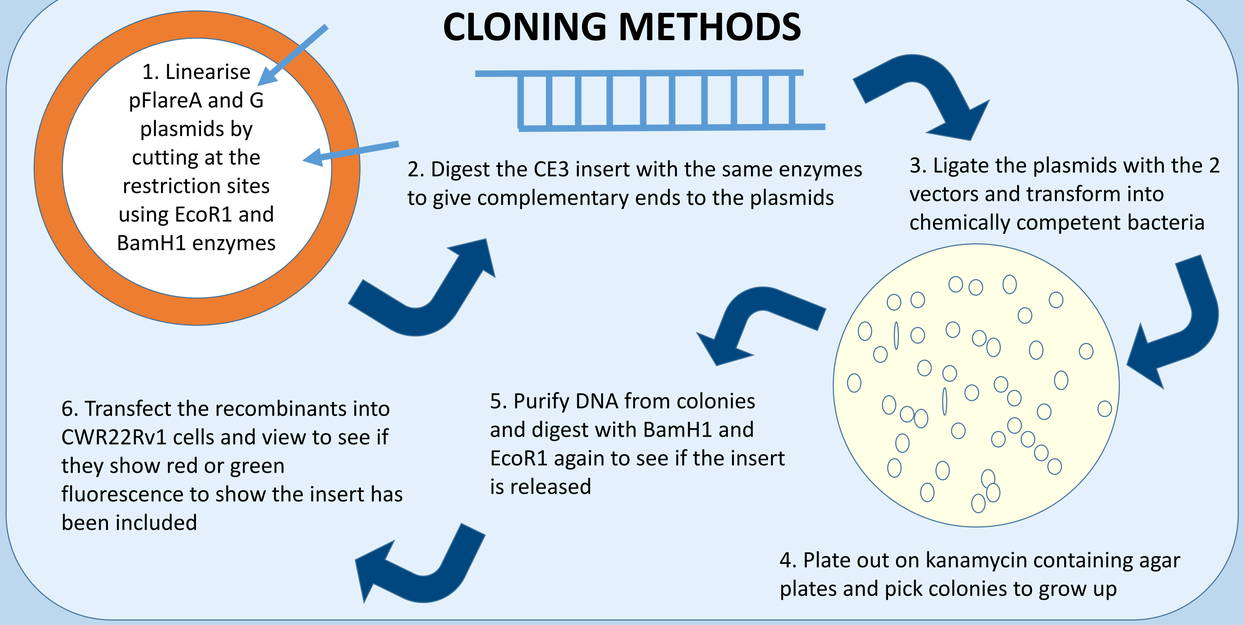
AIMS

- To clone androgen receptor cryptic exon 3 (AR CE3) into pFlareA and pFlareG plasmids
- Transfect CWR22Rv1 cells with the recombinants to show red and green fluorescence, utilising the R/GFP (red/green fluorescent protein)
- Assess the impact of low dose PARP inhibitors (enzyme inhibitors) on AR transcriptional activity

TRANSCRIPTIONAL ACTIVITY METHODS

- Seed down EK cells and let them grow for 48 hours in steroid depleted media
- Treat with the low dosages of BMN (a PARP inhibitor)
- Perform RNA extractions 24 and 48 hours after the drug treatments
- Perform reverse transcriptase reactions on the RNA samples
- Set up qPCR plates incorporating 6 different primers (notably PSA here) to measure the relative expression of different genes in these cells after treatment of BMN
- Compare results to assess the impact of low dosage PARP inhibitor

CLONING METHODS



RESULTS

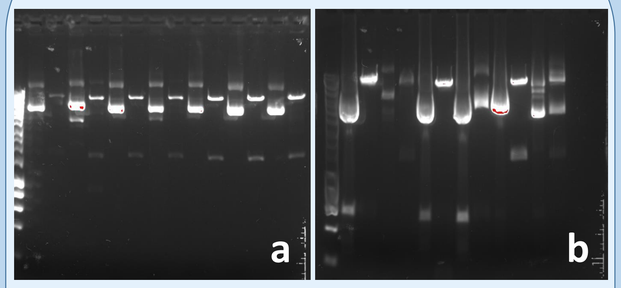


Figure 2a: 1% Agarose gel showing undigested and digested pFlareA plasmids. The lower bands indicate the insert (CE3) showing 6 successful recombinants. *Figure 2b:* 1% Agarose gel showing undigested and digested pFlareG plasmids. Lanes 5 and 11 show successful recombinants where the insert (CE3) has been released upon digestion.

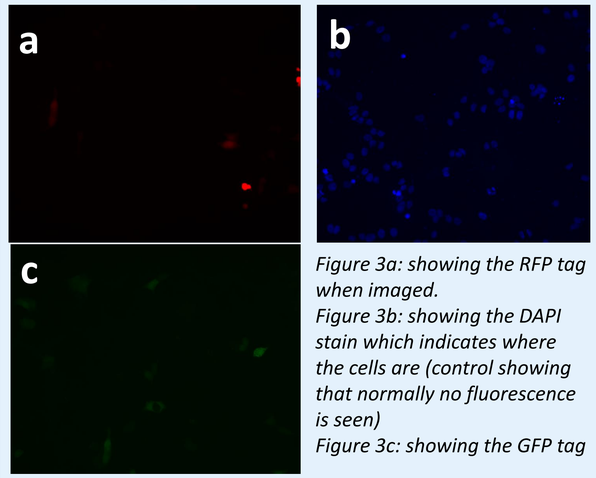


Figure 3a: showing the RFP tag when imaged.
Figure 3b: showing the DAPI stain which indicates where the cells are (control showing that normally no fluorescence is seen)
Figure 3c: showing the GFP tag

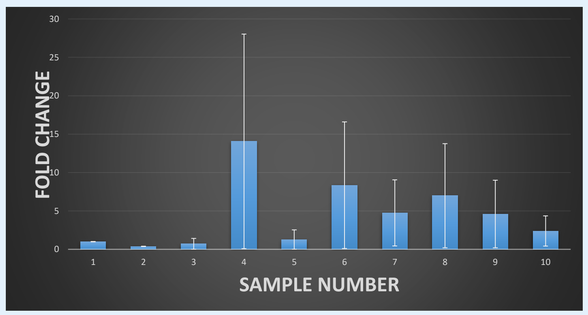


Figure 4: Graph showing the relative expression of PSA after different doses of BMN. Bars 1-5 show 24 hour treatments of increasing concentrations and 6-10 show 48 hour treatments. 1 and 6 show PSA levels in EK cells with no treatment. T test results show results greater than 0.05 and therefore no significant reduction has been seen.

DISCUSSION

- Following digest with EcoR1 and BamH1, it can be seen from *Figure 2a/b* that the insert has been released in both pFlareA and pFlareG, confirmed through sequencing.
- *Figure 3* shows that the system does show the red and green fluorescence and can be taken forward to look at exon inclusion and skipping in cells.
- As AR-Vs are a very important factor in CRPC, it is important to be able to find out what leads to their formation.
- By utilising this system, more can be understood on the formation of AR-Vs, providing new therapeutic targets.
- We also do not know the effect of current drugs on the splicing reaction. Some of the current cancer drug therapies could be leading to alternative splicing and hence CRPC, so if these effects could be identified it could lead to a decrease in CRPC and better initial treatment.
- The efficiency and accuracy of the system is not yet known either, and this is something that would need to be taken into consideration as it may not fully show where a small change in an exon has occurred.
- After treatment of BMN on EK cells, there was no statistically significant result seen in the reduction of transcriptional activity of certain genes (PSA in *Figure 4*).
- In *Figure 4* it can be seen that the standard deviations overlap showing no significant change in expression compared to the housekeeper gene.
- It may be that this type of inhibition works alongside other forms of treatment, and this may be something to look into further.

CONCLUSION

- The CE3 insert was successfully cloned into both vectors, confirmed by the sequencing results.
- Using the red and green fluorescence, it can be visibly seen if the exon is being skipped or included.
- For CRPC, this could provide new therapeutic targets if certain proteins or compounds are found to be involved heavily in the formation of AR-Vs
- There was little to no impact seen on the AR transcriptional activity upon low dosage of a PARP inhibitor.

REFERENCES

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