Optimisation of BaseScope probes for the detection of androgen receptor and its AR-V7 splice variant in prostate cancer tissue

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Introduction

- Prostate cancer (PC) has 47,700 new UK cases every year.
- The androgen receptor (AR) is required to regulate cell growth and survival with initial treatment involving androgen suppression via castration.
- Castration resistant PC (CRPC) may develop, with patients no longer responding to initial treatment.
- Hence an importance to detect AR and splice variants (AR-V) in PC tissue, to develop new treatments.

Aims

- Confirm specificity of AR and AR-V7 targeting RNA probes in a duplex assay for multiplexing with metal conjugated antibodies.
- Optimise BaseScope procedures in different cell types to determine AR and AR-V7 expression levels, to be applied in human tissue.

BaseScope Method

1) Permeabilization- Cells were fixed onto slides and pre-treated to unmask the RNA targeted.

2) Hybridization- AR and AR-V specific double-Z probes were added to hybridize to the target RNA (Figure 1).

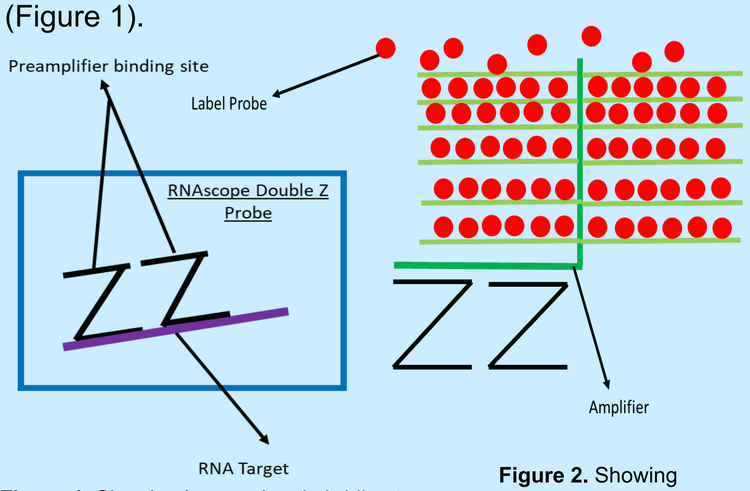


Figure 1. Showing how probes hybridize to target AR and AR-V7 RNA.

Figure 2. Showing amplification of RNA probes.

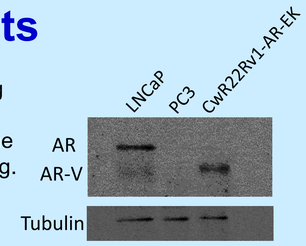
3) Amplification- Detection reagents were used to amplify hybridisation signals, to detect the presence of RNA (Figure 2).

20-40x

4) Visualisation- Using a standard bright field microscope, samples were assessed. Each dot signal represented a single target RNA.

Results

Figure 3. Western blot. Performed using LNCaP, PC3 and CwR22Rv1- AR-EK, probing for AR, AR-V and tubulin. This was to confirm effective knockdowns and demonstrate that the RNA probes do not exhibit non-specific binding. LNCap showed to contain both AR and AR-V, CwR22Rv1-AR-EK showed to contain AR-V only and PC3 lacked both AR and AR-V. Tubulin was used as a normalizer.



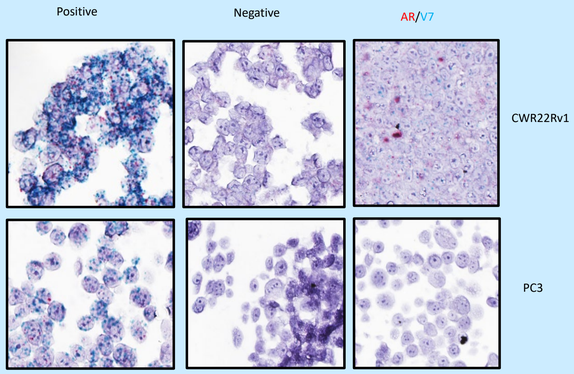
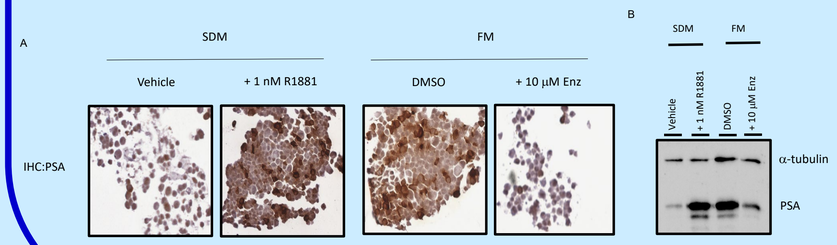


Figure 4. Validation of BaseScope duplex assay. CWR22Rv1 and PC3 cell line pellets were used to optimise the duplex staining assay for full length AR and AR splice variant AR-V7. Positive controls: red = PPIB; blue = POLR2A. Negative controls: red/blue = Bacterial gene DapB.

Figure 5. Immunohistochemistry staining in cell line pellets. (A) IHC for PSA was performed on a panel of cell line pellets treated with either R1881 (1nM) for 24 hours or Enzalutamide for 24 hours to confirm modulation of the AR signalling pathway. (B) Western blotting of protein lysates collected from this batch of cell line samples demonstrates successful stimulation and inhibition of the AR signalling pathway.



Discussion and Conclusion

- The BaseScope procedure was optimised successfully, first using HeLa cell pellets to test positive/negative control probes, detecting both red and green signals.
- This technique was modified to be more cost effective and optimise both coloured signals, without affecting the effectiveness.
- Both AR and AR-V7 were detected using BaseScope RNA probes at different levels in different prostate cancer cell lines, to present these specificities.
- The main difficulty was observing these signals when our own Prostate cell pellets were more dispersed on slides and therefore harder for the slide scanner to detect, although visible under the microscope.
- Signals may now be quantified counting per cell, alongside a semi-quantitative scoring guideline, to give a guide of the level of gene expression of AR and AR-V7 in each cell type.

Future Research

- This technique, now optimised, is able to be applied alongside metal conjugated antibodies to be used in imaging mass cytometry.
- This can then be used to detect AR and AR-V7 as well as related kinases at the RNA level in patient tumour samples, aiming to design an effective treatment against CRPC.

References

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