PARP1 and RAD51 protein expression as biomarkers of homologous recombination status in ovarian cancers

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Introduction

Ovarian cancers can be divided into 2 groups according to their homologous recombination (HR) pathway status.[1] The HR pathway is involved in repairing DNA damage, safeguarding the genetic information of the cells.
This can predict response to standard chemotherapy and novel treatments, as HR-defective tumours seem to respond better to both.

At present, it can only be determined from cultured cells obtained from ascites, which is rarely feasible in a clinical context.
The development of a reliable and clinically feasible test to determine HR status is of key importance.

Our hypothesis was that PARP1 and RAD51 protein expression is a reliable indicator of HR status.
Protein expression was analysed using immunohistochemical (IHC) labelling of tissue microarrays (TMAs) of ovarian cancer.

Optimisation

Prior to labelling the TMAs, we optimised the antibodies using control tissues with different levels of PARP1 and RAD51 expression.

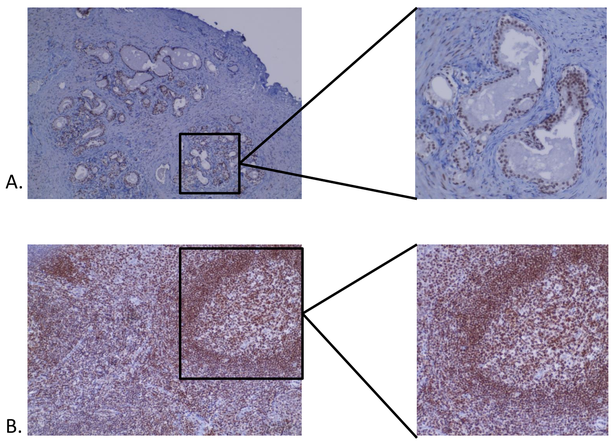

Figure 1- PARP1 labelling in prostate (A) and tonsil (B)tissue, showing a higher magnification region (inset)

Control tissues displayed different levels of labelling, with clear differences observed between the positive glandular cells within the prostate (brown) and the negative stromal cells (blue). The tonsil shows variability in labelling intensity between regions of positive cells

Methodology

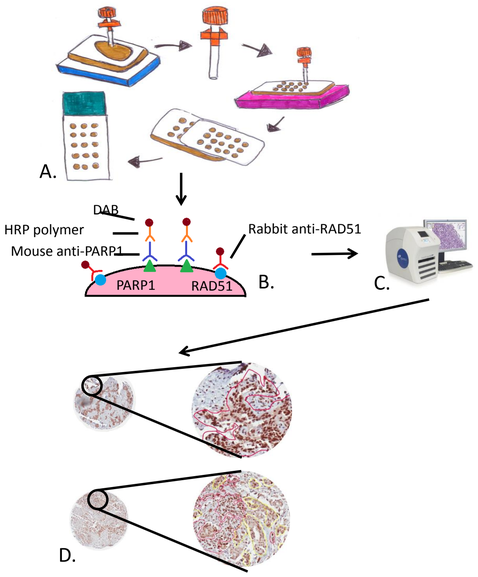

Figure 2- Development and scoring of TMAs

A) In this project we used previously assembled TMAs. The HR status of the tumour cores had been identified using DNA analysis of cultured cells from ascitic fluids.
B) The TMAs were labelled with PARP1 and RAD51 antibodies, using the protocol identified during optimisation. The antibodies are bind to a specific protein; probes are then added to obtain the brown and blue staining.
C) The TMAs were scanned using the Aperio Scanscope System which allows to create digital slides.
D) TMAs were scored using a modified H-score which combined the intensity and the extension of the labelling. Scores ranged from 0 to 24.

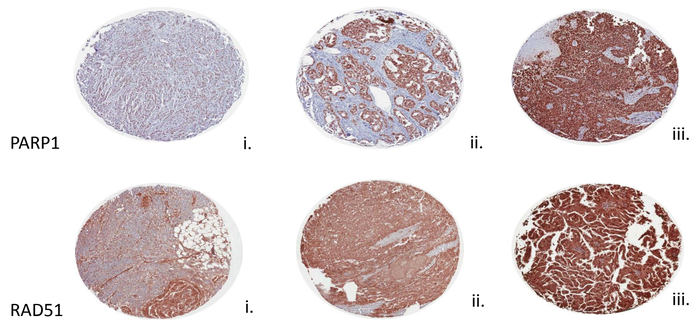

Figure 3- Intensity of labelling for PARP1 and RAD51: weak (i), moderate (ii) and strong (iii).

Results and discussion

We could not identify a correlation between PARP1 and RAD51 expression and HR status.
However, as there are multiple pathways for DNA repair, looking at more factors may produce successful results without compromising clinical feasibility.

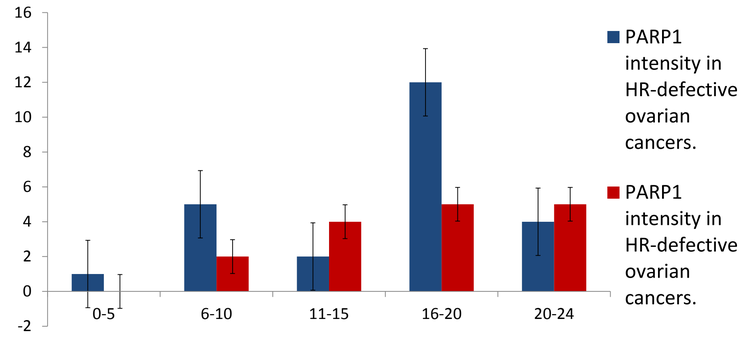

Figure 4- Distribution of scoring in sample TMAs.

Interestingly, we identified a trend between PARP1 expression and progression free survival (PFS): high levels PARP1 seem to link with longer PFS.
This is an important finding as it identifies PARP1 expression as a potential prognostic factor.

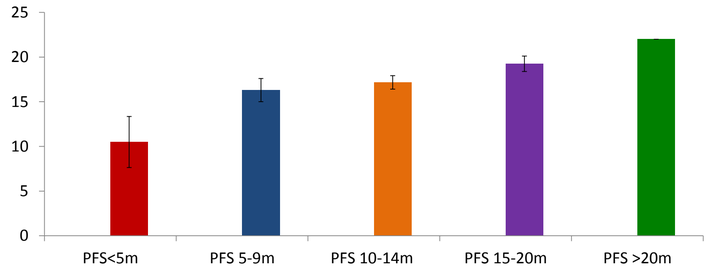

Figure 5- Relation between PARP1 expression and progression free survival (PFS).

Conclusions

•A multifactorial approach should be sought to correlate PARP1 and RAD51 expression with HR status.
•PARP1 expression may have a prognostic value in determining the extent of PFS. Further investigation, inclusive of other factors of influence, should be carried on to validate or disprove such trend.

References
1.Mukhopadhyay, A., et al., *Development of a functional assay for homologous recombination status in primary cultures of epithelial ovarian tumor and correlation with sensitivity to poly(ADP-ribose) polymerase inhibitors.* Clinical Cancer Research, 2010. **16**(8): p. 2344-2351.