Mutational Footprints of DNA Damage and Repair in Homologous Recombination Deficient Human Cells

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Digital poster

Introduction

Mutational signatures are characteristic patterns of somatic mutations that result from DNA damage and repair mechanisms, which can be exploited as biomarkers for patient stratification. For instance, HRDetect uses a combination of signatures to detect one's homologous recombination (HR) status, which could potentially forecast PARP inhibitor (PARPi) sensitivity.

To date, large-scale cancer sequencing studies have linked the loss of BRCA1, BRCA2, PALB2 and RAD51C proteins to the single-base substitution pattern SBS3 and microhomology-mediated deletions. However, whether perturbing the other players in the HR pathway is associated with any mutational signatures remain elusive.

In this study, we addressed this gap by establishing ground truth HRd mutational signatures, encompassing single-base substitutions and small indels, in an isogenic cell line model (hTERT-RPE1) by first knocking out four canonical HR genes (*BRCA1*, *BRCA2*, *PALB2*, *RAD51C*). We subsequently investigated the presence of these signatures in KOs of putative HR genes (see **Figure 1**) alongside their PARPi sensitivities.

Our study provides insights into the nuanced relationship between HRd mutational signatures and PARPi sensitivity, underscoring the potential of leveraging these signatures as biomarkers for the stratification of patients eligible for PARPi therapies.

Method

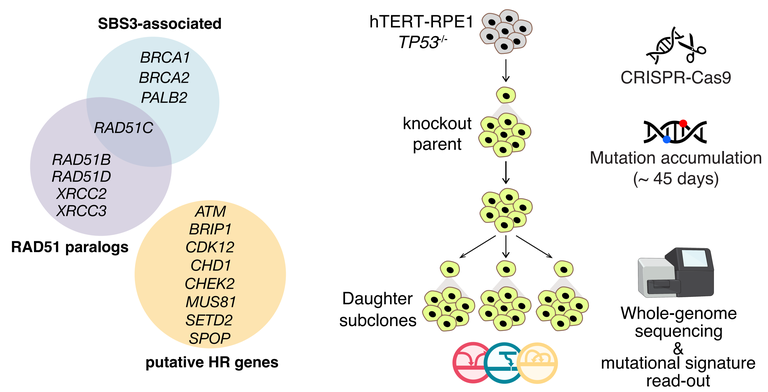

Figure 1. Schematic diagram of mutation accumulation experiment

Results

All canonical HRd genotypes produced elevated substitution and indel burdens. Their substitution and indel profiles closely resembled each other and cancer-derived HRd signatures, namely SBS3 and InD6 (cosine similarity >0.85), and were distinct from the control[1,2]. Genotype-specific signatures were derived by subtracting the control background from individual profiles (**Figure 2**). These SBS and indel signatures were combined respectively to constitute our ground truth experimental HRd-associated SBS and indel signatures, termed eSBS.HRd and eInD.HRd respectively (**Figure 3**).

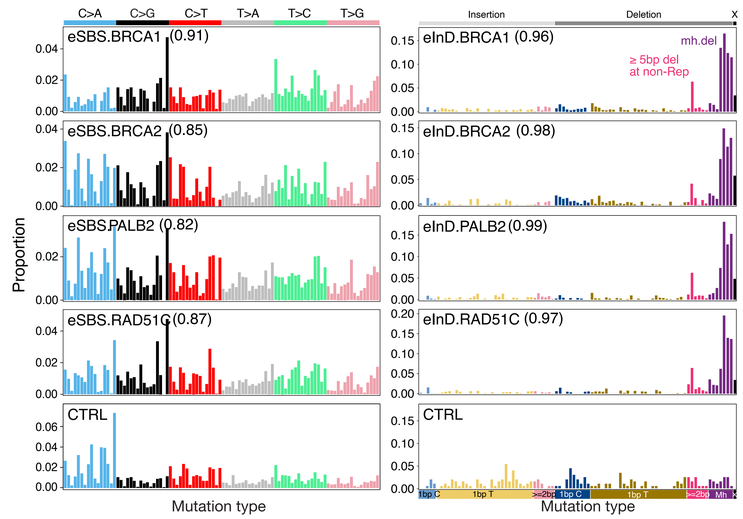

Figure 2. Genotype-specific substitution and indel mutational signatures of canonical HR KO genotypes (brackets indicate cosine similarity compared to cancer derived HRd signatures SBS3 and InD6 respectively)

We next fitted eSBS.HRd and eInD.HRd to investigate their presence in 16 HRd genotypes (and control), including all RAD51 paralogs and genes that have been suggested to exhibit putative functions in the HR pathway (**Figure 3**). RAD51 paralogs KOs (except for *RAD51B*$^{-/-}$) demonstrated consistently high levels of eSBS.HRd and eInD.HRd in proportions comparable with canonical HR gene KOs. Apart from *SETD2*$^{-/-}$, all putative HR gene KOs showed lower proportions of eSBS.HRd than canonical HR and RAD51 paralog KO genotypes. However, none of these putative genes demonstrated eInD.HRd.

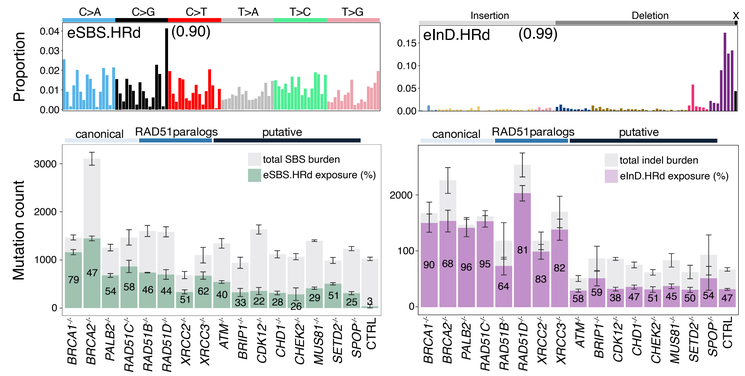

Figure 3. Experimental HRd single-base substitution and indel mutational signatures (brackets indicate cosine similarity compared to cancer derived HRd signatures SBS3 and InD6 respectively)

We applied several algorithms to detect HRd on this data (**Figure 4**). HRDetect and CHORD were closely correlated with each other and strongly associated with the canonical and RAD51 paralog KO genotypes[3,4]. Sigma produced a substantial number of false positives. Whereas Myriad exhibited both false negative and false positive predictions for HRd.

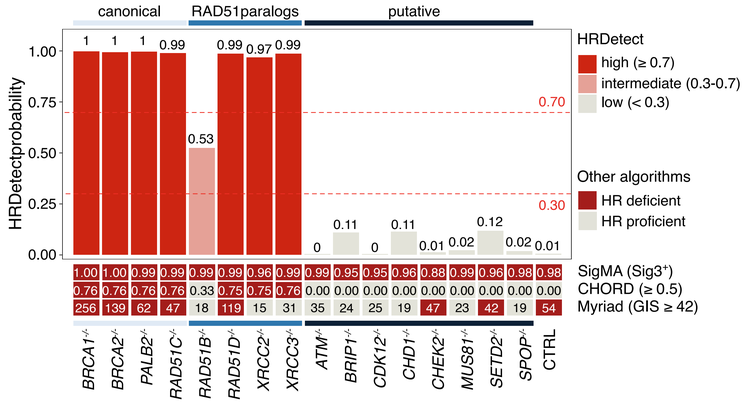

Figure 4. Comparison between HRDetect and other algorithms in the detection of HRd genotypes in RPE cells

Intriguingly, HRd may not be sufficient to forecast PARPi sensitivity. All genotypes that were predicted as HRDetect high and intermediate were sensitive to PARPi (**Figure 5**). On the other hand, KO of a plethora of putative HR genes also sensitised the cells to PARPi. Genotypes can be HRDetect low yet more sensitive to PARPi than *BRCA1*$^{-/-}$ and *BRCA2*$^{-/-}$ (e.g. *CDK12*$^{-/-}$, *ATM*$^{-/-}$). This is because, despite all involved in different levels of the HR pathway, the various classes of mutational signatures (SBS, indel and rearrangement) in other HRd genotypes were not always prominent as observed under the biallelic loss of canonical HR genes.

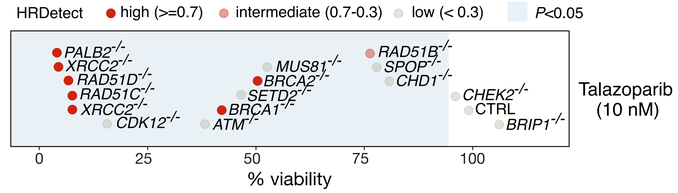

Figure 5. Differential PARPi sensitivities of HRd genotypes (6-day exposure)

Conclusion & Future Directions

We present the ground truth of mutational signatures generated through experimental KO of canonical HR genes (i.e. *BRCA1*, *BRCA2*, *PALB2*, *RAD51C*), and contrast these to a series of RAD51 paralogs and putative HR gene KOs.

New genes that present signatures which are similar to canonical HR gene KOs now include *RAD51D*, *XRCC2* and *XRCC3*.

HRDetect identified all canonical and RAD51 paralogs KO genotypes as HR deficient.

HRd status captures only some cases that exhibit PARPi sensitivity. Other biomarkers need to be considered in order to capture therapeutic vulnerability to PARPi.

Acknowledgement

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For further information about mutational signatures, please visit SIGNAL website by scanning the QR code on the left (https://signal.mutationalsignatures.com).

References

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