Investigating Replication Repair Deficient Tumours Using Genetic Drivers in Mouse Models

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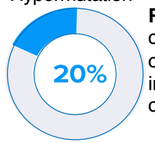
Background

Hypermutation



RRD = most common cause of hypermutation in childhood cancers

DNA replication repair machinery

polymerase proofreading + mismatch repair (MMR)

CMMRD is a RRD cancer predisposition syndrome

Patients with CMMRD frequently succumb to high-grade gliomas

Somatic mutation

Differences in clinical outcomes + response to therapy

Animal models needed to better characterize RRD gliomas

Hypothesis

- RRD gliomas group into 3 distinct groups: **MMRD+*POLE***, **MMRD+*TP53***, **MMRD+*IDH1***
- Each RRD glioma group will display differential tumour developmental behaviour and vulnerability to distinct therapeutic modalities.

Methods

RRD Glioma Model Design & Downstream Analysis

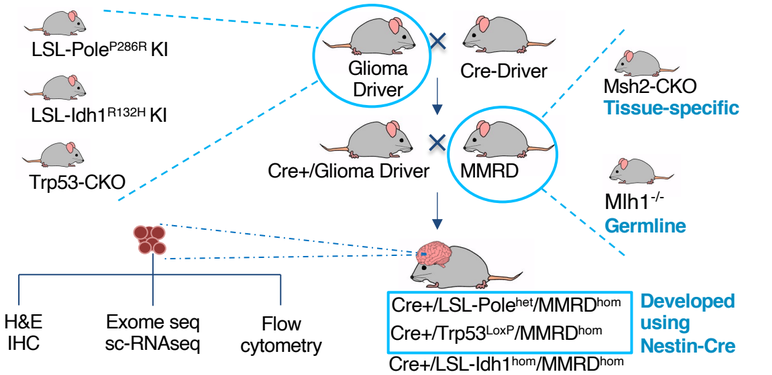


Figure 1. Experimental design of RRD glioma models. Nestin-Cre (NC) mice are combined with glioma driver mouse lines, LSL-Pole^{P286R}, LSL-Idh1^{R132H}, or Trp53^{LoxP} mice, resulting in expression of P286R mutation on Pole, expression of R132H on Idh1, or the complete loss of p53, respectively, specifically in the brain, and then subsequently crossed with MMRD mouse lines: Msh2^{LoxP} and Mlh1$^{-/-}$ mice, to establish the representative RRD glioma models. Brain tumours are analyzed by 1) histopathology and staining for glioma markers, 2) submitted for NGS, and 3) tumour infiltrating lymphocytes (T-cells) examined via flow cytometry.

Results

Pole/MMRD Mice Robustly Develop Hindbrain Tumours

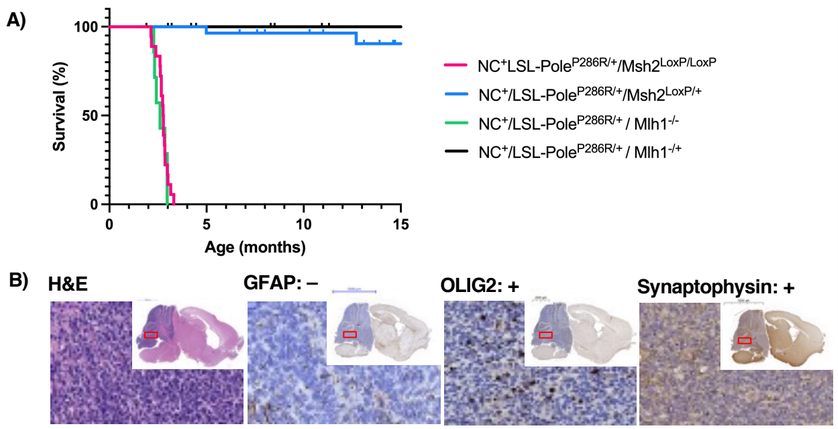


Figure 2. Survival and histopathology findings in Pole/MMRD mice. A) NC+/LSL-Pole$^{P286R/+}$/Msh2$^{LoxP/LoxP}$ (n=18) and NC+/LSL-Pole$^{P286R/+}$/Mlh1$^{-/-}$ (n=7) mice both have reduced survival compared to NC+/LSL-Pole^{P286R}/MMRD^{het} mice. All mice except NC+/LSL-Pole$^{P286R/+}$/MMRD^{hom} mice succumb to hindbrain tumours. **B)** Cerebellar section in NC+/Pole^{P286R}/MMRD mice consistently stain –ve for GFAP, and heterogeneously +ve for Olig2 and synaptophysin, morphologically classifying as a medulloblastoma (MB).

Trp53/MMRD Mice Display Heterogenous Brain Tumour Development

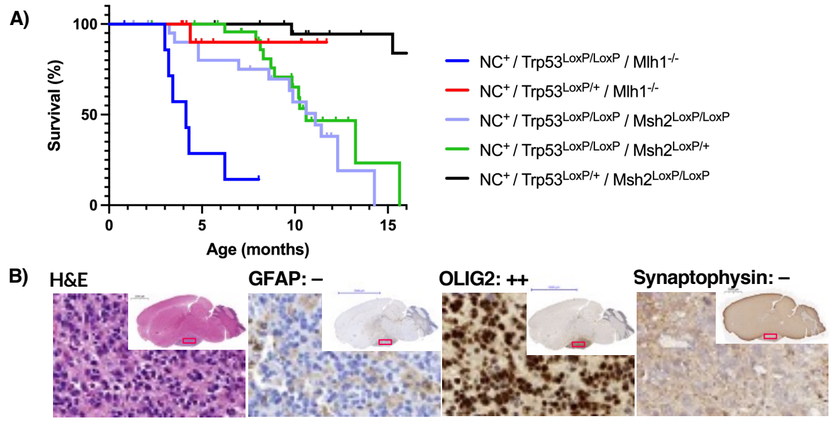


Figure 3. Survival and histopathology findings in Trp53/MMRD mice. A) NC+/Trp53$^{LoxP/LoxP}$/Mlh1$^{-/-}$ mice (n=6) succumb to brain tumours with the worst survival (avg=4.1 months). NC+/Trp53$^{LoxP/LoxP}$/Msh2 mice (n=28) develop brain tumours at different kinetics (avg. survival=10.8 months). Brain tumours in both p53 models arise in different regions (hindbrain, forebrain, olfactory bulb) classifying morphologically as glioma-like or MB-like. **B)** IHC of a forebrain section in NC+/Trp53$^{LoxP/LoxP}$/Msh2$^{LoxP/LoxP}$ tumour indicate –ve GFAP and synaptophysin, and a strong +ve Olig2 stain, classifying as a high-grade glioma subtype (tumour type/location not seen in Pole/MMRD mice).

Results

TMB in RRD Mouse Tumours Mimic Human RRD Tumours

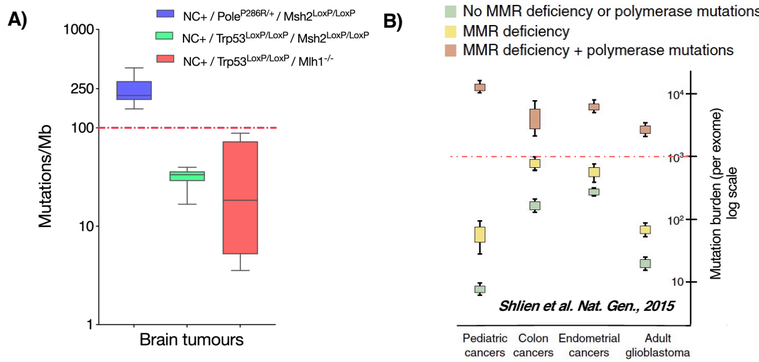


Figure 4. Genomic profile of RRD mouse tumours mirror human RRD cancers. A) NC+/LSL-Pole$^{P286R/+}$/Msh2$^{LoxP/LoxP}$ tumours are consistently ultra-hypermutant (n=7, avg. 243Mut/Mb), whereas NC+/Trp53$^{LoxP/LoxP}$/Msh2$^{LoxP/LoxP}$ are hypermutant (n=7, avg. 31.8 Mut/Mb). **B)** Human MMR+POLE (RRD) tumours are ultra-hypermutant, whereas MMR only brain tumours are hypermutant, representative of the observed results in RRD mouse brain tumours.

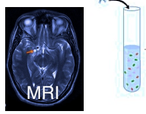
Conclusions

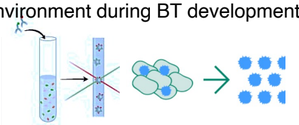
- NC+/LSL-Pole^{P286R}/MMRD (Mlh1$^{-/-}$ and Msh2^{LoxP}) develop ultra-hypermutant cerebellar tumours.
- NC+/Trp53^{LoxP}/Msh2^{LoxP} develop heterogenous hypermutant brain tumours that differ in morphology and are hypermutant.
- NC+/Trp53^{LoxP}/Mlh1$^{-/-}$ succumb to brain tumours earlier than p53/Msh2 mice. TMB needs to be explored more.
- Genomic similarity of mouse and human RRD tumours suggests usefulness of models to study + stratify RRD glioma groups.

Future Directions

1. Develop novel MMRD+Idh1 model (Olig2-Cre driver)

2. Uncover evolution of tumour immune microenvironment during BT development





Flow cytometry (TILs)

3. Test the use of immunotherapy (i.e. ICI)

immune-competent mice

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