Quantification of loss of heterozygosity in Fischer *p53* knockout rats using ddPCR

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Hypothesis: Fischer *p53*$^{+/-}$ knockout rats develop tumors through loss of heterozygosity (LOH), and the mechanism of LOH can be determined by accurately quantifying gene copy number through the use of droplet digital PCR (ddPCR).

Background

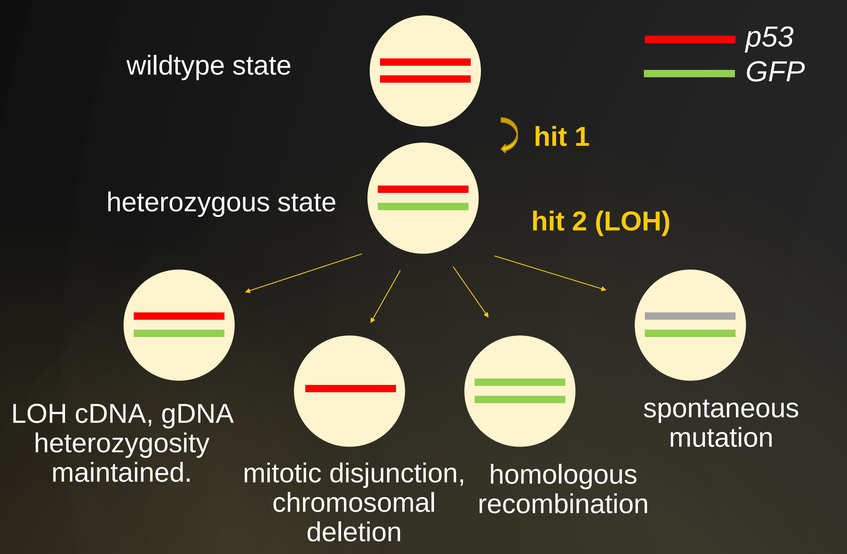


Figure 1: Knudson's 2 hit hypothesis for cancer development, with 4 potential mechanisms for LOH.

***p53* knockout rats:**

- germline competent rat ES cells
- variable phenotype between strains (fig 2)
- better models for some cancers than mice (fig 3).

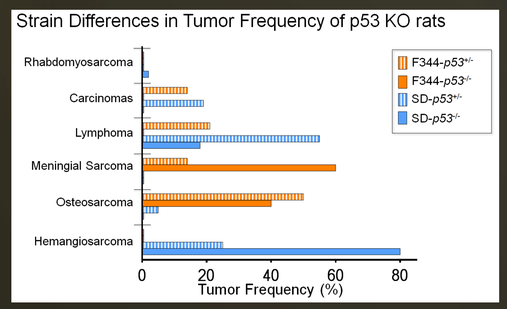
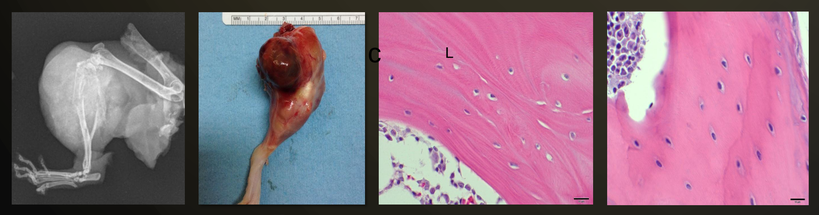


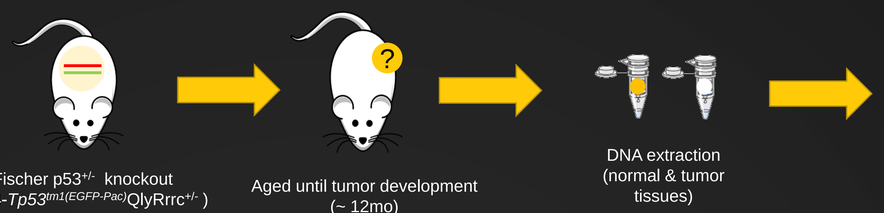
Figure 2: Tumor variation in *p53*$^{+/+}$ and *p53*$^{+/-}$ Sprague Dawley and Fischer rats



A B C D

Figure 3: Radiograph (A) and gross (B) depiction of osteosarcoma from *p53* knockout rat. Histology from bone of rat (C) and mouse (D). Like humans, rats form true lamellae (L), which mice lack.

Materials and methods

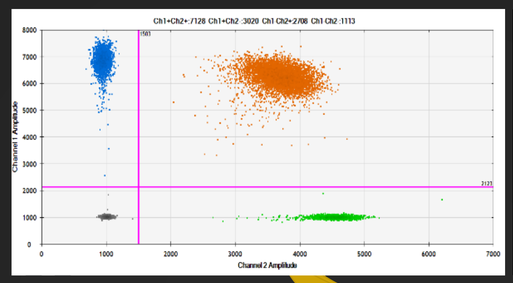


Fischer p53$^{+/-}$ knockout (F344-*Tp53*$^{tm1(EGFP-Pac)}$QlyRrrc$^{+/-}$)

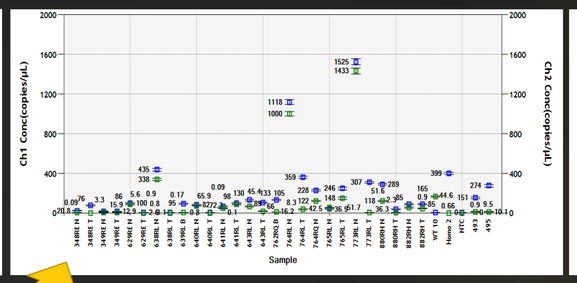
Aged until tumor development (~ 12mo)

DNA extraction (normal & tumor tissues)

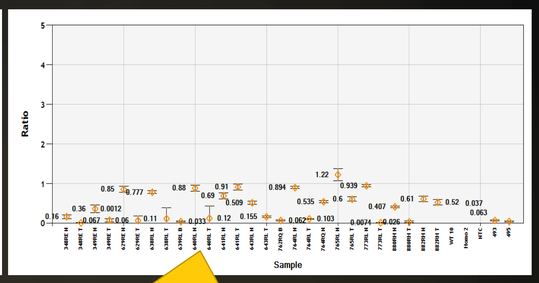
Droplet digital PCR (ddPCR): nanodrop partitioning technology.



Raw ddPCR results

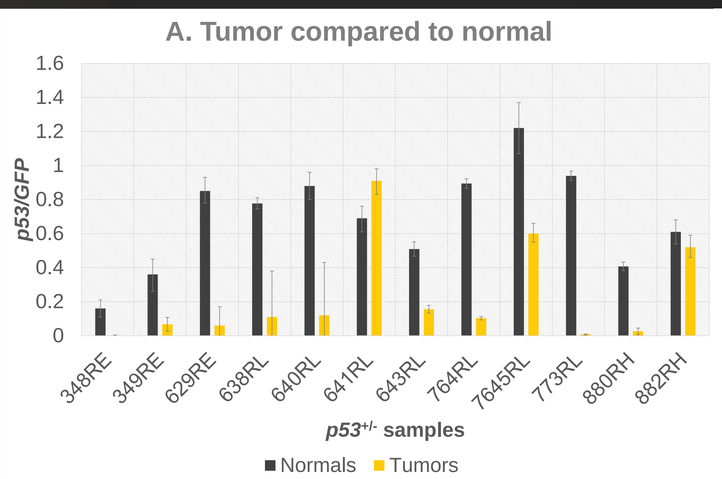


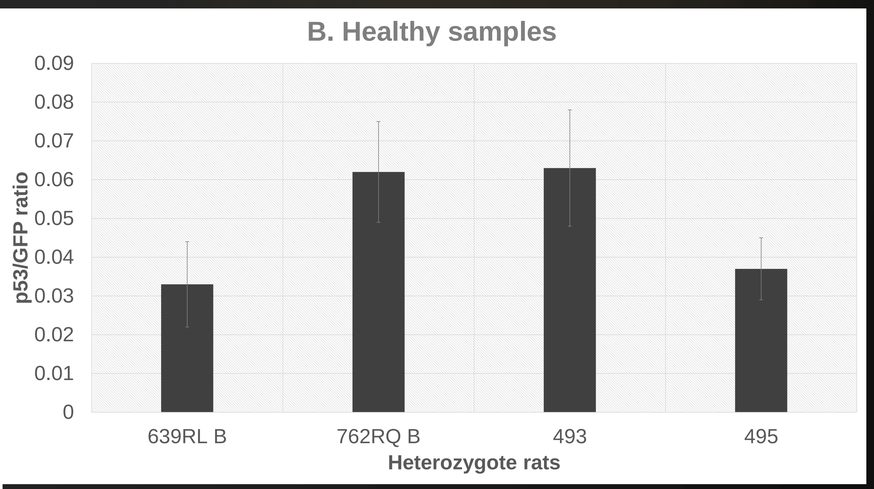
GFP and *p53* concentrations

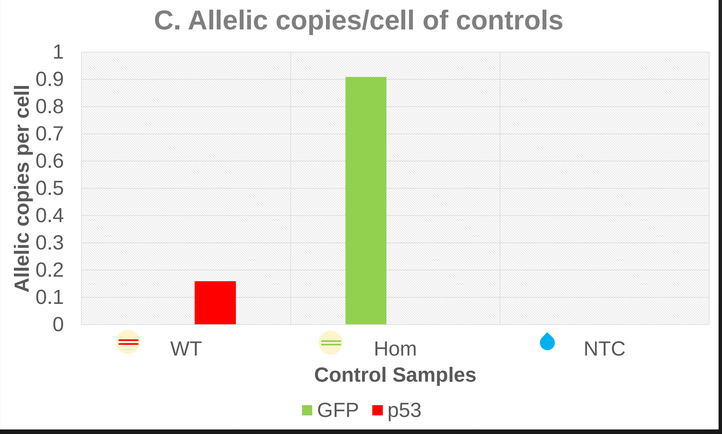


p53/GFP

Results







Figures A-C: LOH shown in 10 of 12 tumor samples when compared to normal of that animal (A). *p53/GFP* expected to be ~1 in normal tissues, but lower values found even in blood of clinically healthy individuals (B) may explain discrepancies. Calculated allelic copies per cell (C) in controls show further refinement of assay necessary for accurate gene number quantification.

Summary

1. Developed LOH ddPCR assay.
2. Demonstrated LOH in Fischer *p53*$^{+/-}$ rats, consistent with other cancer models.
3. ddPCR has a increased sensitivity compared to qPCR, but further refinement is needed for accurate quantification of copy number.

Acknowledgements

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