Mitochondrial DNA mutations reduce rates of *N-Myc* induced tumorigenesis.

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Introduction

N-Myc is a protooncogene. The mitochondrial DNA (mtDNA) mutator mouse model harbors a point mutation (D257A) in DNA polymerase gamma, which results in a proofreading deficiency and the accumulation of somatic mtDNA mutations (Kujoth et al., 2005; Trifunovic et al. 2004). Through a retrograde response, mtDNA can influence metabolic pathways, gene expression and epigenetics. Immunofluorescent imaging can be used to monitor changes in methylation and acetylation of histones (Greer and Shi, 2012).

Methods

Progenitor cells from wild-type (W) and PolG mtDNA mutator mice (P) were infected with a retroviral vector containing *N-Myc* (N) or YFP (Y) through a spinning transduction. Cells were cultured in methocult and liquid culture assays and used for immunofluorescent imaging on a software called Imaris. Cells were also transplanted into lethally irradiated mice and the recipients were monitored for tumor growth.

Results

- PolG progenitors infected with *N-Myc* (PN) showed reduced colony numbers upon serial replating in methocult and lower tumor incidence *in vivo.* Reduced tumor incidence also led to higher survival rates in the PN mice.
- A change in epigenetics occurred between WN and PN groups in the H3K27Me3 and H3K4Me3 methylation markers.

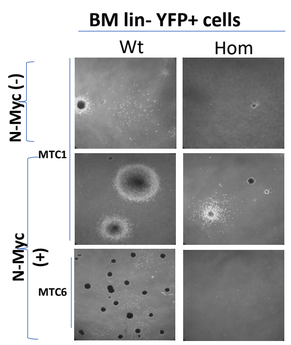

Figure 1. Depiction of the *in vitro* methoculture. WN cells increase in tumor incidence over time as seen from MTC1 to MTC6. PN cells have significantly lower tumor incidence across time.

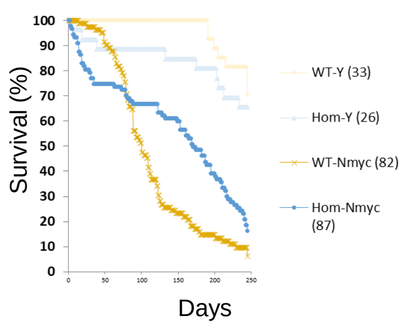

Figure 2. This graph was taken from the *in vivo* experiments. It measured the survival of mice lethally irradiated after injection with the mtDNA mutations.

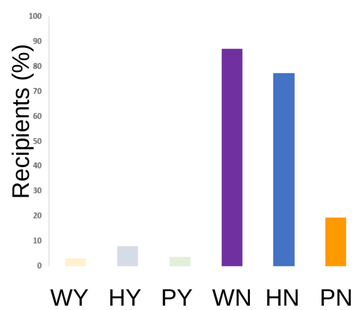

Figure 3. This graph was taken from the *in vivo* experiments. It depicts the tumor incidence rates among the *in vivo* models.

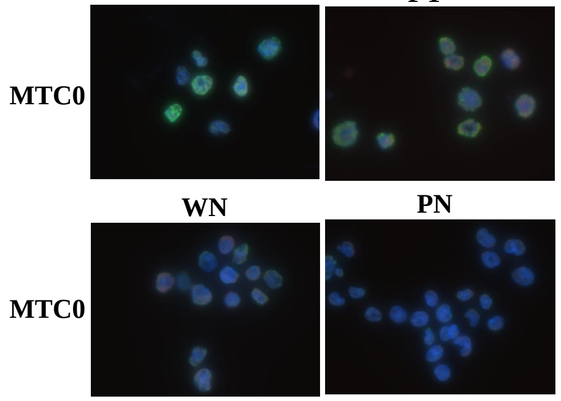

Figure 4. A sample of the images taken from the immunofluorescent staining used to quantify the data. A color change (expression of H3K4Me3 and H3K27Me3 markers) can be seen between the genotypes indicating changes in epigenetics.

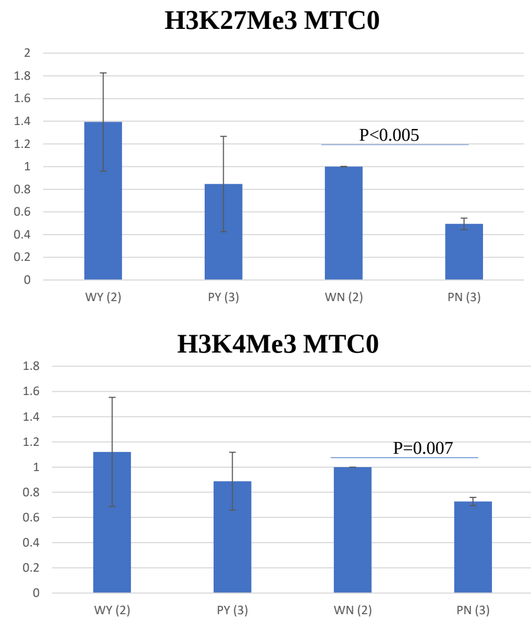

Figure 5. Depicts analysis of the changes in the expression of the H3K4Me3 and H3K27Me3 markers between genotypes. All change was standardized based on the WN group.

Conclusion

- The introduction of *N-Myc* in wild-type bone marrow cells drives proliferation and tumorigenesis (Figure 1).
- Tumor development *in vitro* and *in vivo* is reduced by the high mtDNA mutation burden in the mtDNA mutator mice .
- The H3K27Me3 was upregulated while the H3K4me3 was suppressed in PN at MTC0 suggesting epigenetic changes decreased tumorigenesis (Figure 4, 5).
- Future research would investigate the contribution of mtDNA mutation associated metabolic and epigenetic changes on tumorigenesis.

Literature Cited

Greer EL, Shi Y. 2012. Histone methylation: a dynamic mark in health, disease and inheritance. *Nat Rev Genet.* 13(5):343-57.

Kujoth GC, Hijona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, Hofer T, Seo AY, Sullivan R, Jobling WA, Morrow JD, Van Remmen H, Sedivy JM, Yamasoba T, Tanokura M, Weindruch R, Leeuwenburgh C, Prolla TA. 2005. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science.* 309(5733):481-4.

Trifunovic A, Wrendenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, Bohlooly-Y M, Gidlof S, Oldfors A, Wibom R, Tornell J, Jacobs HT, Larsson NG. 2004. Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature.* 429(6990):417-23.