Defective Homologous Recombination in Aged Mice

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INTRODUCTION

Age is one of the biggest risk factors for cancer, a disease which is often characterized by an accumulation of DNA damage[1]. Therefore, we wanted to test if the DNA repair machinery responsible for mitigating DNA damage degrades with age.

Homologous recombination (HR) is one of the least error-prone DNA repair pathways and is the primary pathway utilized during meiosis. Thus, we used meiotic homologous recombination as a paradigm for studying age-related degradation of HR machinery.

The fundamental purpose of meiosis is to segregate chromosomes accurately. This requires a minimum number of crossovers, which are the products of HR. To initiate HR, intentional double-stranded breaks (DSBs) are introduced into the chromosomes by an enzyme called SPO11.

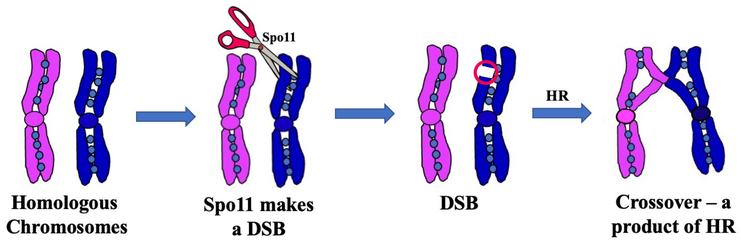

Figure 1: Formation of a Crossover

Meiotic cells lacking a crossover between each pair of homologous chromosomes fail to form viable embryos in most scenarios. Therefore, the cell works to maintain the required number of crossovers despite fluctuations in DSB numbers in a process termed "Crossover Homeostasis[2]." We tested whether crossover homeostasis was maintained in aged male mice. Since SPO11 is a suicide enzyme, we used an animal heterozygous for the *Spo11* allele to reduce the number of DSBs and test for crossover homeostasis.

Understanding such age-dependent changes in DNA repair machinery provides a logical framework for developing unified interventions to counteract age-related dysfunction and diseases such as cancer.

HYPOTHESIS

We hypothesized that in aged mice, crossover homeostasis is defective. The rationale for this hypothesis is that crossovers were reduced in WT old mice along with an apparent reduction in DSB numbers, suggesting a potential defect in crossover homeostasis. This phenomenon was not observed in WT adult mice[3]. Therefore, we expected to see a disruption of crossover homeostasis in aged *Spo11* heterozygotic mice but not in adult *Spo11* heterozygotic mice.

METHODOLOGY

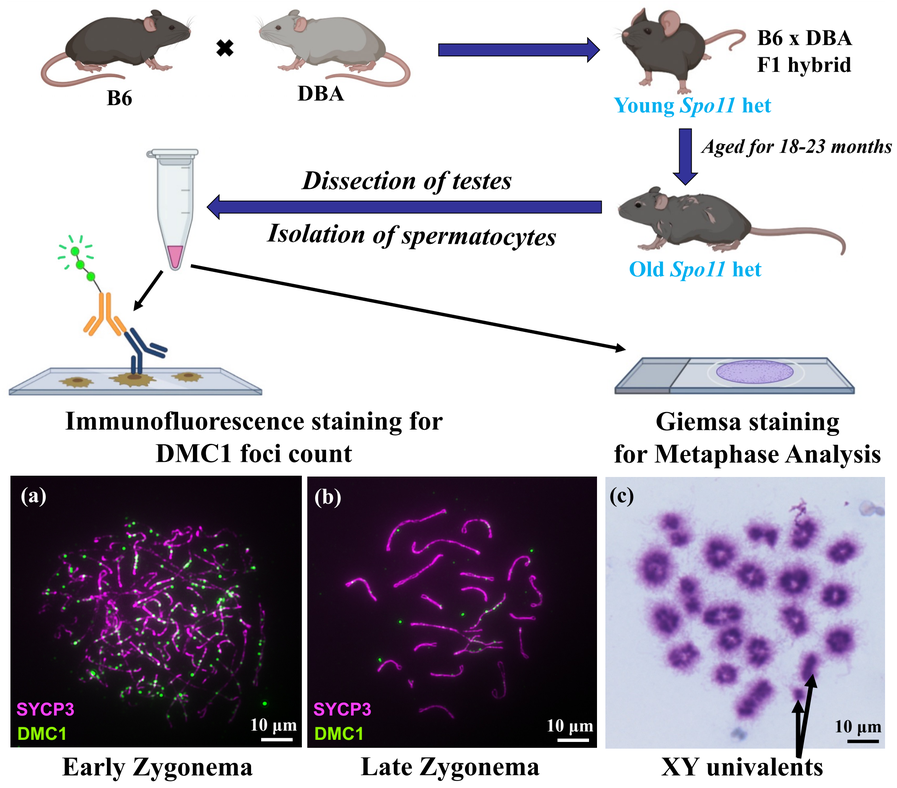

Figure 2: Schematic of basic methods used in project[4].
(a) & (b) DMC1 foci are a representative indicator of DSBs and appear in the second stage of Prophase termed Zygonema, which can be further divided into substages: Early Zygonema (a) and Late Zygonema (b). SYCP3 is a chromosomal axis protein.
(c) Unpaired chromosomes, called univalents (characteristic of the disruption of Crossover Homeostasis), can be detected using Metaphase spreads. XY univalents are shown in (c).

RESULTS

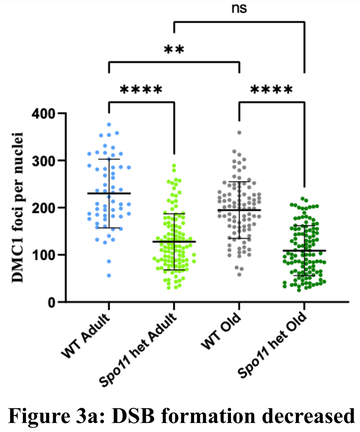

Figure 3a: DSB formation decreased in *Spo11* het animals
Consistent with earlier reports[2], decreasing SPO11 leads to a reduction in DSB formation in both Adult and Old mice.

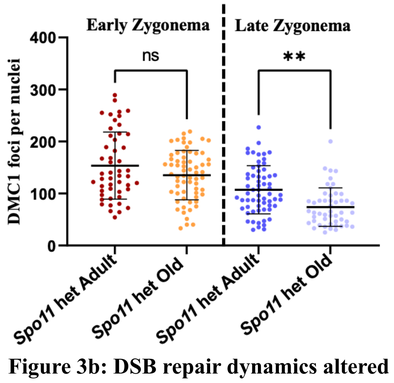

Figure 3b: DSB repair dynamics altered in Old mice
Old mice show a decreased DMC1 foci in Late Zygonema suggesting that DNA repair dynamics are altered.

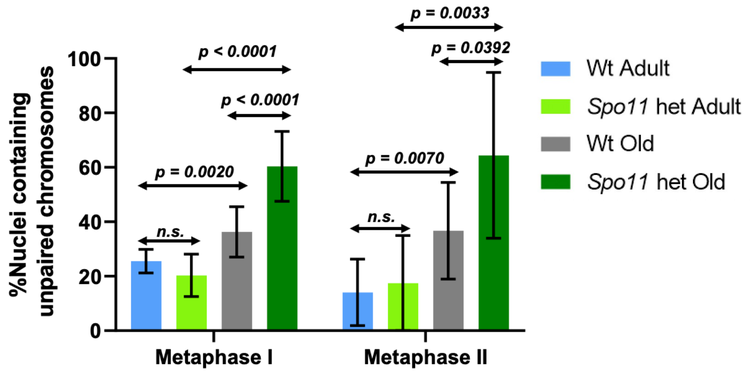

Figure 4a: Crossover homeostasis is defective in Old mice
Both WT Old and *Spo11* het Old mice have significantly more univalents than either WT Adult or *Spo11* het Adult mice. Removing a copy of the *Spo11* gene causes crossover defects only in Old mice and not in Adult mice. Together, this demonstrates a defect in crossover homeostasis in Old mice. Further this aneuploidy was observed after the first chromosome segregation, indicating that these cells make it past the first meiotic checkpoint and could produce aneuploid gametes.

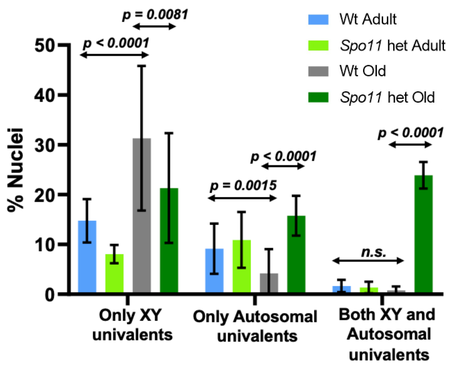

Figure 4b: Drivers of Aneuploidy
In the case of WT Old mice, the aneuploidy was driven by the unpairing of the XY chromosomes, whereas in *Spo11* het Old mice, aneuploidy was driven by the unpairing of both XY chromosomes and autosomes, which indicates a more severe phenotype.

CONCLUSIONS

We have demonstrated that Homologous Recombination is defective during meiosis in old mice. Specifically, we saw that crossover homeostasis, one of the fundamental properties of meiosis, is affected. Discounting mutants that directly perturb the crossover repair pathway, this is the first ever observation of a defect in crossover homeostasis in any organism yet. The mouse model was aged naturally, and the genetic perturbation didn't affect the DNA repair machinery, thus allowing our observations to become transferrable to old somatic cells and correspondingly old humans (70-90 years old). Considering that this is typically the age when DNA mutational load causes diseases such as cancer, this research is relevant for the development of therapeutic interventions to tackle the root causes of such age-related dysfunction.

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