Deciphering Roles of Bloom Syndrome Helicase (Blm) in Genome Stability

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itcms integrated training in cancer model systems

BACKGROUND AND INTRODUCTION

(1) Mitotic Crossovers (mitCOs) resulting from Homologous Recombination (HR) repair of DNA are highly detrimental to cells, leading to potential loss of heterozygosity (LOH), genome instability, and cancer[1,2].

(2) Bloom Syndrome Helicase (*Drosophila*: *Blm*; Human: *BLM*) has been shown to cause **increased** spontaneous mitotic crossover rates when absent in the *Drosophila* male germline, mammalian cells, and Human Bloom Syndrome cells[3-7].

(3) Accurate interpretation of mitCO mechanisms in multicellular organisms was previously difficult due to obfuscation of heteroduplex DNA tracts via canonical and short-patch mismatch repair (cMMR and spMMR). Complete MMR knockout (KO) has **only** been acheived in *Drosophila*, leading to a new model for meiotic crossovers[8].

(4) Gain in copy number of BLM has been observed in several cancers, suggesting potential alternative roles for Blm in these cancers.

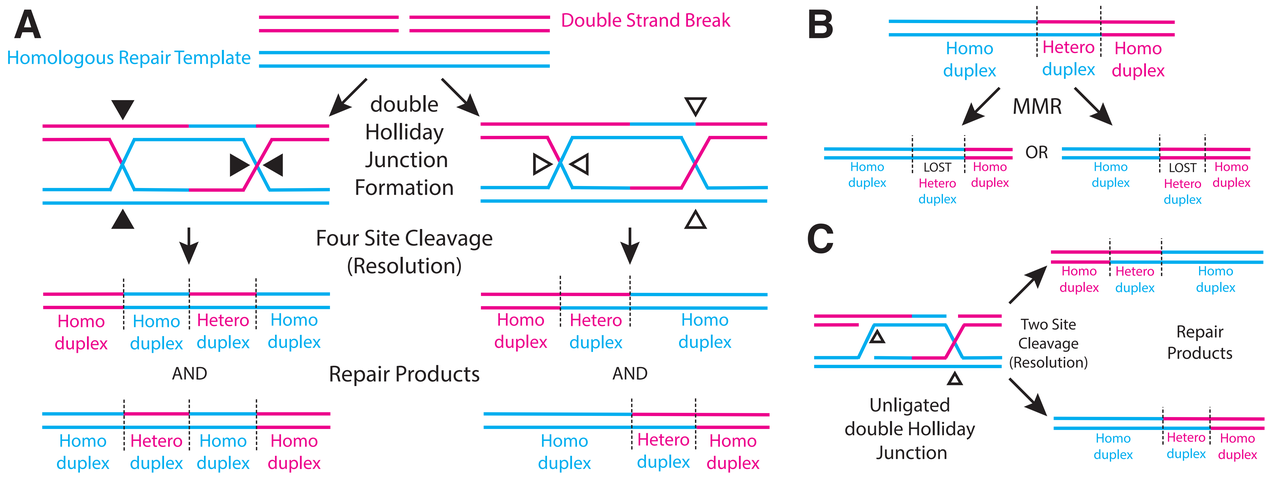

Figure 1. (A) Double Strand Break (DSB) repair mediated by Homolgous Recombination (HR) repair. Double Holliday Junction (dHJ) intermediates are one model for how HR repairs DSBs. Differing dHJ cleavages at four sites (filled vs. open arrowheads) result in distinct hetero- and homoduplex DNA arrangements in repair products. Some products have four distinct DNA duplexes surrounding the repaired DSB (left), while others have three (right). These arrangements help determine where a DSB originated and how it was repaired. **(B)** Mismatch Repair (MMR) converts informative heteroduplex DNA to homoduplex DNA, making finding where a DSB originated and how it was repaired difficult to interpret depending on how the conversion occurs. This is done via canonical and short-patch (cMMR and spMMR) pathways. **(C)** A meiotic crossover (CO) model suggests use of an unligated dHJ and two site cleavage (resolution) rather than four site cleavage based on KO of MMR and the meiotic CO products observed[8]. Such a model could be applicable to mitotic COs (mitCOs).

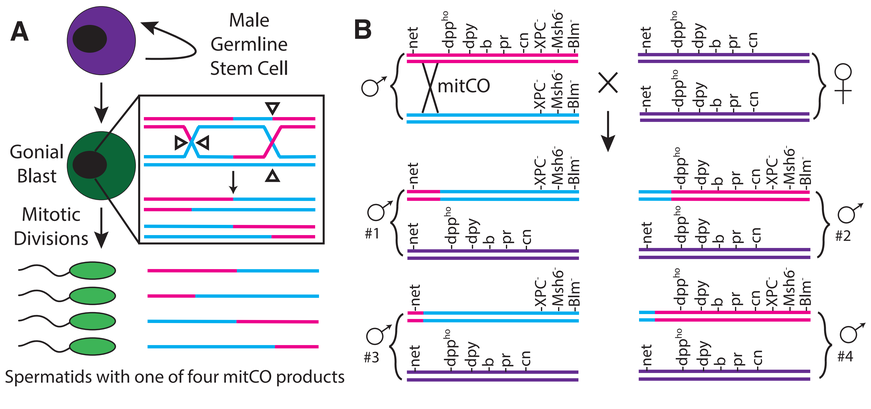

Figure 2. (A) Depiction of *Drosophila* male germline divisions. A mitCO during the Gonialblast division (callout box) results in spermatids with one of four products. **(B)** By mating males heterozygous for net-cn and homozygous for spMMR and cMMR (XPC$^-$ and Msh6$^-$, respectively) knockout (KO) and Blm KO to net-cn females, mitCOs (e.g. between net and dpp^{ho}) are recovered by screening male progeny for reciprocal product arrangements indicated by net, dpp^{ho}, dpy, b, pr, and cn recessive markers (e.g. #1-4). Precise mitCO sites are then mapped via Illumina or Oxford Nanopore whole genome sequencing of one male, followed by Sanger sequencing to map remaining reciprocal products.

HYPOTHESES

(1) Resolution of double Holliday Junctions (dHJ) will prevail as the mechanism for Blm KO spontaneous mitCOs.

(2) Alternative and/or secondary mechanisms may apply to mitCOs, such as one described for meiosis[8].

(3) Blm overexpression will not cause overgrowth or tumor-like phenotypes in normal epithelia.

(4) Blm gain of copy number in certain cancers will promote growth through alleviation of replication fork damage, and prevent complete collapse of genome stability through HR.

PRELIMINARY DATA AND RESULTS

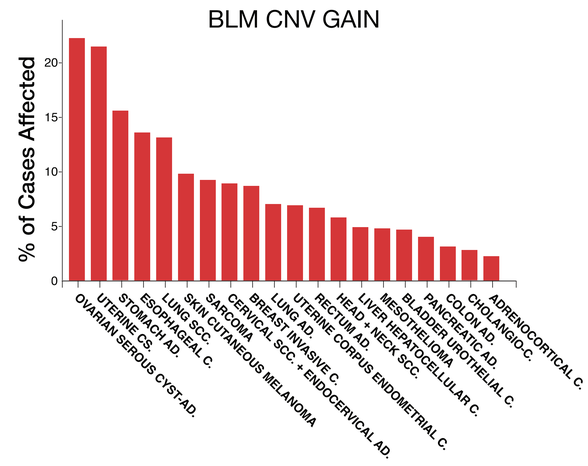

Figure 3. Percentanges of Various Cancers Affected by BLM Copy Number Variation (CNV) Gain. While BLM has been previously implicated in both Bloom Syndrome and other cancers by a loss of function, recent data from The Cancer Genome Atlas (TCGA) instead show many cancers gain in BLM copy number, suggesting a potential alternative role for BLM in these cancers.

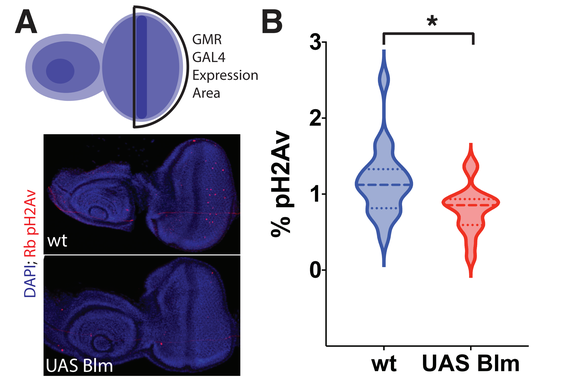

Figure 4. (A) Schematic of GMR GAL4 driver expression area and representative images of wt and UAS Blm (Blm overexpression) *Drosophila* imaginal eye disc epithelia. GMR drives expression to the right of the second mitotic wave cells (dark band) in the larger eye disc, indicated by the black semi-oval outline. Representative images of wt and UAS Blm discs stained with DAPI (blue) and with the DSB marker rabbit (Rb) α-pH2Av (*Drosophila* H2A.X; red). **(B)** Violin plot of percent pH2Av signal in GMR GAL4 expression area shows UAS Blm decreases DSBs compared to wt (*p< 0.05, unpaired t-test). Dashed line = median; dotted line = 1st and 3rd

CONCLUSIONS

(1) Blm overexpression in *Drosophila* imaginal eye discs reduces the amount of DSBs, indicating that copy number gain (and presumably overexpressed) *BLM* could reduce the number of DSBs in cancer to promote basal genomic stability and persistence.

CITATIONS AND ACKNOWLEDGEMENTS

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