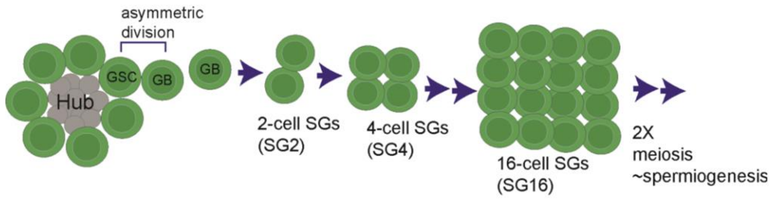
Roles of Histone Inheritance in Regeneration and Tumorigenesis

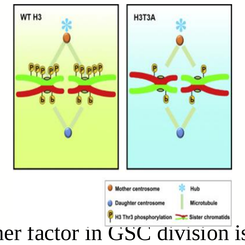
Vedansh Patel and Mayu Inaba
Department of Cell Biology, University of Connecticut Health, Farmington, CT

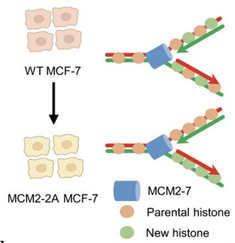
Introduction

Drosophila testes are maintained through the use of stem cells at a location of the tip of testes called the niche or the hub. There are usually 8-12 germline stem cells that surround the hub and then divide asymmetrically into 1 GSC that remains at the hub and 1 gonialblast. The GB further divides, eventually undergoing spermatogenesis.



During this division of GSCs, materials in the cell are divided asymmetrically, including histones. As the histones divide into 2 cells, the preexisting histone remains with the GSC and the newly formed histones go to the GB. Threonine 3rd on Histone H3 is known to be phosphorylated during mitosis to differentiate pre existing and new histones. With H3T3A transgenic flies, there is a mutation where Threonine is replaced by an Alanine that prevents phosphorylation, thus hindering asymmetric distribution of histones. This is known to cause defects in GSCs and can cause tumors in the *Drosophila* testes.

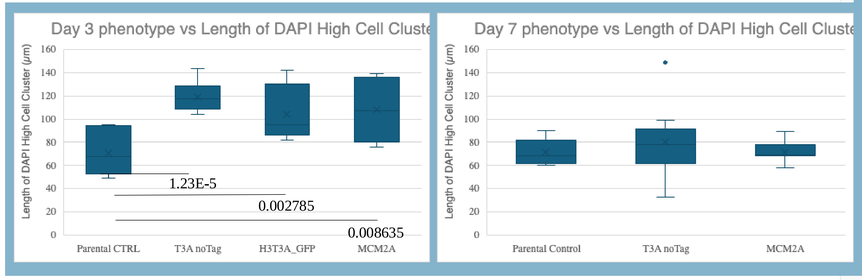




Another factor in GSC division is the MCM2 complex, which is responsible for proper histone sorting in DNA replication. When MCM2 is phosphorylated, it is available to divide the old and new histones so that each of the new DNA strands can keep the memory of the old DNA strand histone modification. With a MCM2A mutation, the complex is not able to divide the histones in a way that will keep the memory of the old DNA, thus resulting in problems with cell division.

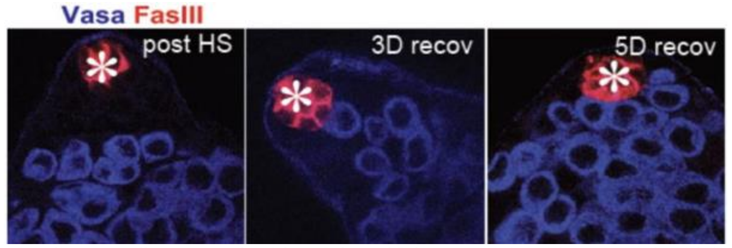
Question

It is thought that tissue regeneration is implicated in the formation of cancer. We use the *Drosophila* testes and their simple anatomical model to induce regeneration, or differentiation, of the germline stem cells through the heat shock method to investigate whether stem cell regeneration and histone inheritance can influence tumorigenesis.



Methods

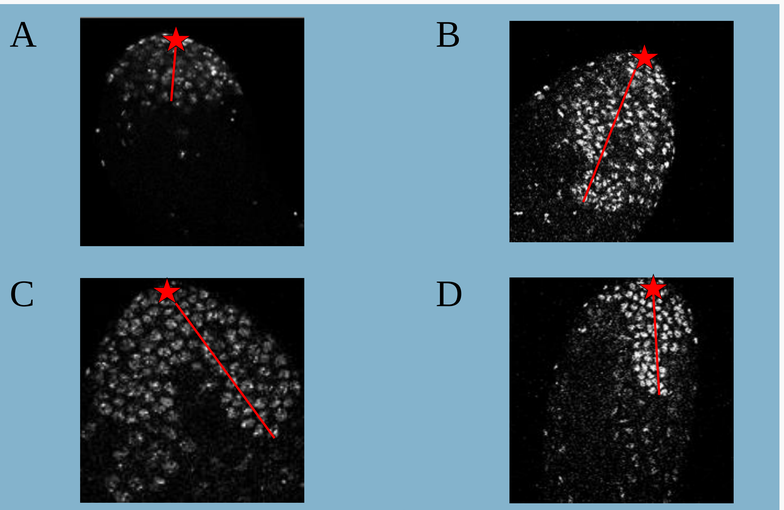
Heat shock was used in this experiment to induce a loss of GSCs. All groups were placed in 37 C water for 30 minutes at a time for a total of 6 times. In between the heat shocks they were kept in 29 C. These heat shocks would take place starting Tuesday evening continuing until Friday morning for each morning and evening. On day 3 and day 7 after the last heat shock, the GSCs would recover as seen in the image below and the flies were dissected to retrieve their testes.



Germline stem cells recover at the niche at 3 day and 5 day recovery (asterisk represents the niche)

Once the testes had been harvested, they underwent immunofluorescence staining. They were placed in 4%FA solution and rotated for 30 minutes at room temperature. The 4%FA was removed from the tube and the testes were washed with 1000mL and rotated for 20 minutes, and repeated 2 more times. A primary antibody solution was made using Fas3, VASA, and 1B1 in 3% BSA at a 1:20 dilution factor. 100ml of the solution was placed in each vial after removing the PBST and incubated overnight at 4 C. The solution was then removed and the testes were washed 3 times with PBST and incubated at room temperature for 30 minutes. The secondary antibody solution was the prepared using mouse Cy3 and rat Cy5 in a 3% BSA solution with a 1:200 dilution factor. 100mL was added and the solution was incubated for 2 hours at room temperature. The solution was then removed and washed 3 times with PBST and incubated for 30 minutes. The PBST was removed and 1 drop of mounting liquid with DAPI was added to each vial. The testes were then mounted onto slides to be seen under the microscope.

Results



Images above show the DAPI staining of the *Drosophila* testes that were harvested after the day 3 recovery after heat shock. Note the uniform distance in the control (A) compared to the longer growths in the MCM2A (B), T3A noTag (C), and T3A GFP (D) samples. The star represents the hub and the farthest point of the DAPI signal was measured with a straight line. The 2 graphs on the left show the difference in length between the phenotypes on Day 3 and Day 7. The P-values are listed on Day 3 to show the significant difference in length between the control and the transgenic flies.

Summary

- Interestingly, the H3T3A and MCM2A mutations enhanced the size of these DAPI clusters compared to the parental control
- The clusters increased as seen on Day 3, but then decreased after Day 7
- Normally, the DAPI signal would be seen in undifferentiated cells such as the GSCs and GBs. However, the heat shock seems to have caused some of the early stage differentiated cells to acquire undifferentiated properties such as the high DAPI signal, making the cluster of high DAPI cells larger than that seen in the pre heat shock testes

Discussion

It is possible that the regeneration of the germline stem cells after the heat shock is able to influence the increase of the size of the DAPI cell cluster. Tumorigenesis consists of the uncontrolled growth of cells that can result from mutations and causing cancers. When the stem cells leave the niche and come back after the recovery period, they could possibly have undifferentiated states, such as the DAPI high cells seen in this experiment. It is possible that this results in the stem cells obtaining factors seen in pre-tumorous conditions, yet we do not know the mechanism by which DAPI-high cells increase around the niche and the possible consequence correlated to tumorigenesis.

Our next step involves looking at GFP expression and its variation in the germline stem cells after a heat shock treatment. In nos-GFP flies, GFP is seen expressed in the GSCs of their testes. With MCM2A flies, it is seen that there is variation in GFP expression in the GSCs before heat shock treatment. However, after the heat shock is seems as if the variation decreases and the expression is more uniform. It is thought the MCM2A mutation can influence the histone inheritance and therefore create variation where some cells have high expression and some have low expression. After the heat shock, the GSCs leave the niche and come back during recovery. There may be a bias towards cells that have a high expression of GFP to return to the niche, as they may contain more stem cell-like properties, which is why we may see a more uniform expression of GFP after recovery. We are also looking to monitor flies that are kept in 29 C continuously for 2 weeks, as this will induce more loss and regeneration cycles. This will be a method to amplify the effects seen from disturbances in histone inheritances and its influence of tumorigenesis. We will test the H3T3A and MCM2A phenotypes in both experiments.

References

Xie J, Wooten M, Tran V, Chen BC, Pozmanter C, Simbolon C, Betzig E, Chen X. Histone H3 Threonine Phosphorylation Regulates Asymmetric Histone Inheritance in the Drosophila Male Germline. Cell. 2015 Nov 5;163(4):920-33. doi: 10.1016/j.cell.2015.10.002. Epub 2015 Oct 29. PMID: 26522592; PMCID: PMC4636931.

Inaba, M., Yamashita, Y.M. (2017). Evaluation of the Asymmetric Division of *Drosophila* Male Germline Stem Cells. In: Buszczak, M. (eds) Germline Stem Cells. Methods in Molecular Biology, vol 1463. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-4017-2_3

Ratajczak, M.Z., Bujko, K., Mack, A. *et al.* Cancer from the perspective of stem cells and misappropriated tissue regeneration mechanisms. *Leukemia* 32, 2519–2526 (2018). https://doi.org/10.1038/s41375-018-0294-7

Gianna Maggiore1, and Hao Zhu1. Relationships Between Regeneration, Wound Healing, and Cancer. December 18, 2023. Vol. 8:177-197. https://doi.org/10.1146/annurev-cancerbio-062822-123558

Wenger, A., Biran, A., Alcaraz, N. *et al.* Symmetric inheritance of parental histones governs epigenome maintenance and embryonic stem cell identity. *Nat Genet* 55, 1567–1578 (2023). https://doi.org/10.1038/s41588-023-01476-x

Ridwan, S.M., Twillie, A., Poursaeid, S. *et al.* Diffusible fraction of niche BMP ligand safeguards stem-cell differentiation. *Nat Commun* 15, 1166 (2024). https://doi.org/10.1038/s41467-024-45408-7

Tian, C., Zhou, J., Li, X. *et al.* Impaired histone inheritance promotes tumor progression. *Nat Commun* 14, 3429 (2023). https://doi.org/10.1038/s41467-023-39185-y

Acknowledgements

Thank you to Dr. Mayu Inaba and Burak Bener for helping with this project and to the Office of Undergraduate Research for funding this opportunity.

vedansh.patel@uconn.edu
(860)385-0108

Lab website: https://health.uconn.edu/germline-stem-cells/