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Chemical Ablation of Stem Cells

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Introduction

Planarians are an amazing model system for studying regeneration due to their large population of stem cells. A critical technique in studying stem cell dynamics is to eliminate stem cells through exposure to gamma or X-ray radiation[1,2]. By eliminating the stem cells of planarians, experimental phenotypes that would otherwise be masked by the stem cells can be studied. But these radiation techniques require expensive equipment and upkeep. The goal of this project was to develop a cheap alternative to kill stem cells by using the cell cycle inhibitor hydroxyurea. Hydroxyurea acts on the S phase of the cell cycle leading proliferating cells to arrest and stop synthesizing DNA (Fig.1)[3].

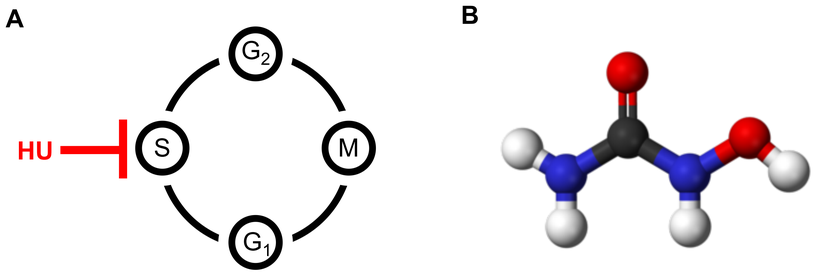

Figure 1. Hydroxyurea (HU) Mechanism of Action and Structure. (A) Cell cycle diagram showing where hydroxyurea acts. (B) Chemical Structure of hydroxyurea molecule[4].

Approach

To eliminate the stem cells in *Schmidtea mediterranea* animals were soaked in differing concentrations of hydroxyurea. The concentrations (5 mM, 10 mM, and 40 mM) being tested were adapted from previous research by Gambino[1]. Hydroxyurea was diluted in planarian salts before each soak. Once a sublethal concentration was established animals were soaked in varying concentrations for 24H, cut, and allowed to soak for 4 days during their regeneration process. Mitotic index staining (MIS) was used to assess whether there was a reduction in cell proliferation (Fig. 2, 3, and 5). Fluorescent in situ hybridization (FISH) using stem cell marker *Smed-H2B* was used to assess whether stem cells were lost following hydroxyurea treatment (Fig. 4, 5).

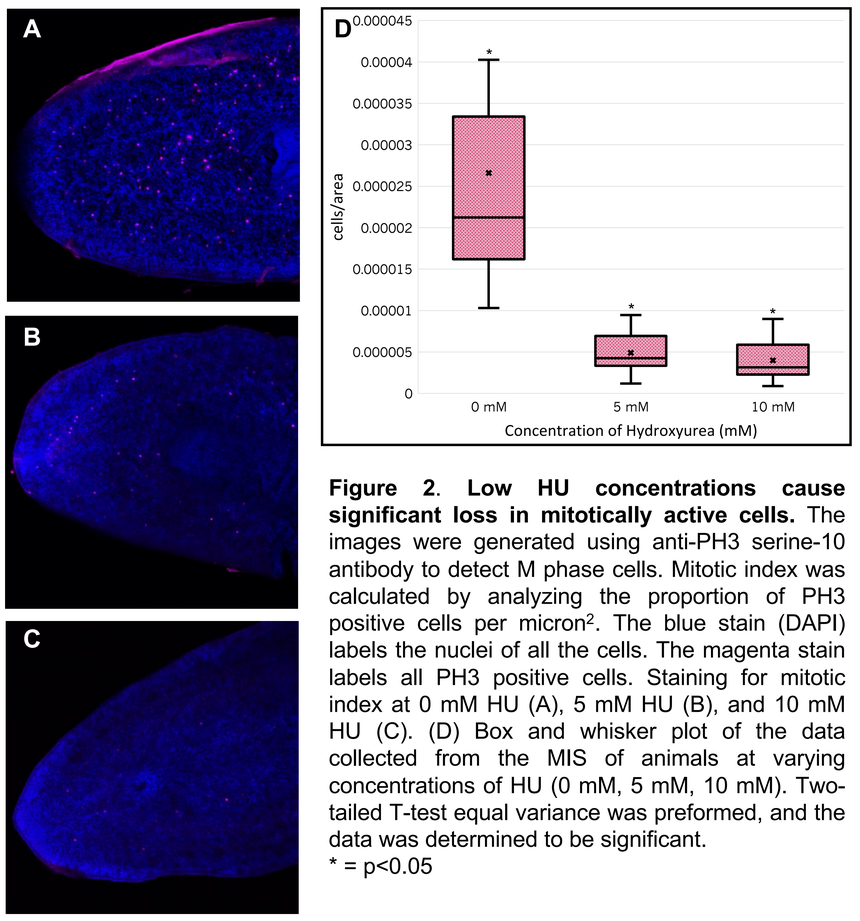

Figure 2. Low HU concentrations cause significant loss in mitotically active cells. The images were generated using anti-PH3 serine-10 antibody to detect M phase cells. Mitotic index was calculated by analyzing the proportion of PH3 positive cells per micron^2. The blue stain (DAPI) labels the nuclei of all the cells. The magenta stain labels all PH3 positive cells. Staining for mitotic index at 0 mM HU (A), 5 mM HU (B), and 10 mM HU (C). (D) Box and whisker plot of the data collected from the MIS of animals at varying concentrations of HU (0 mM, 5 mM, 10 mM). Two-tailed T-test equal variance was preformed, and the data was determined to be significant.
* = $p<0.05$

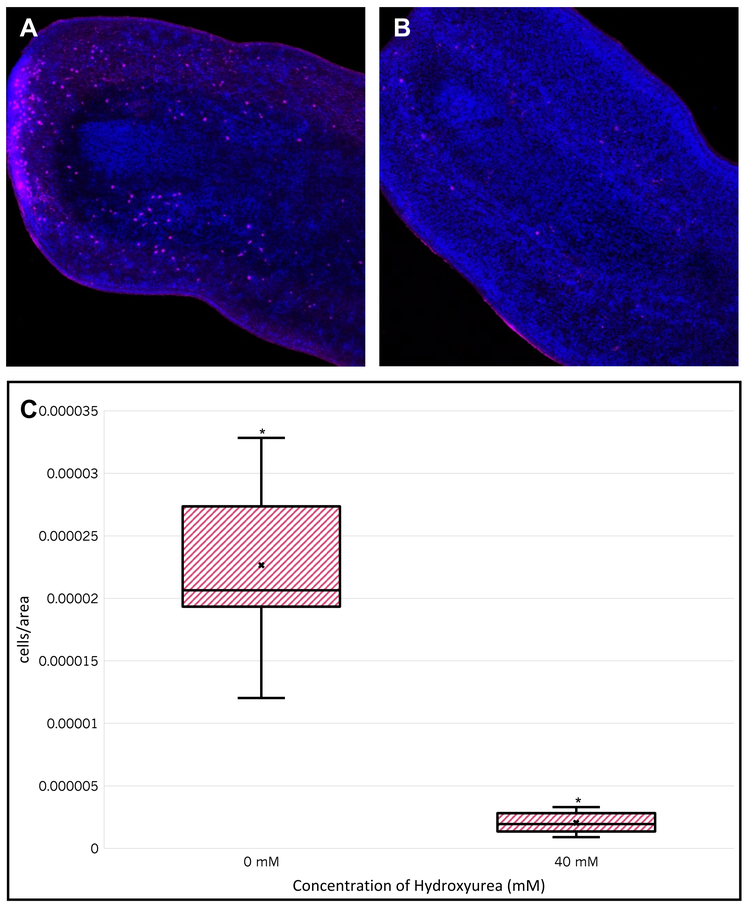

Figure 3. High HU concentrations cause significant loss in mitotically active cells. Staining for mitotic index at 0 mM HU (A), and 40 mM HU (B). (C) Box and whisker plot of MIS.

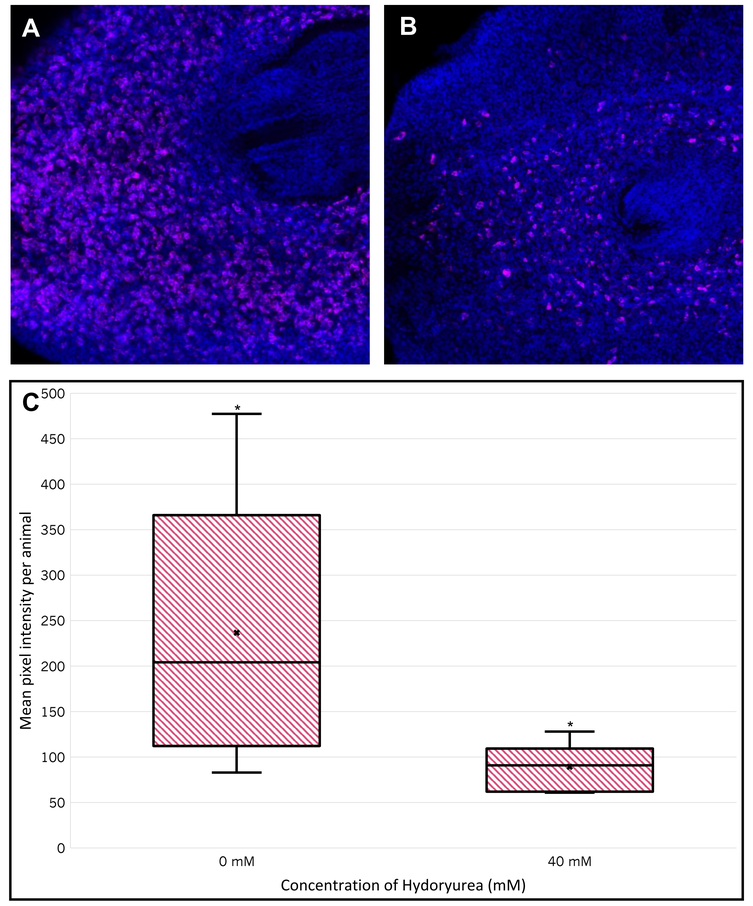

Figure 4. High HU concentrations causes significant loss in stem cell pool. FISH for the stem cell marker *Smed-H2B* at 0 mM HU (A) and 40 mM HU. (B). (C) Box and whisker plot of mean of pixel intensity per animal.

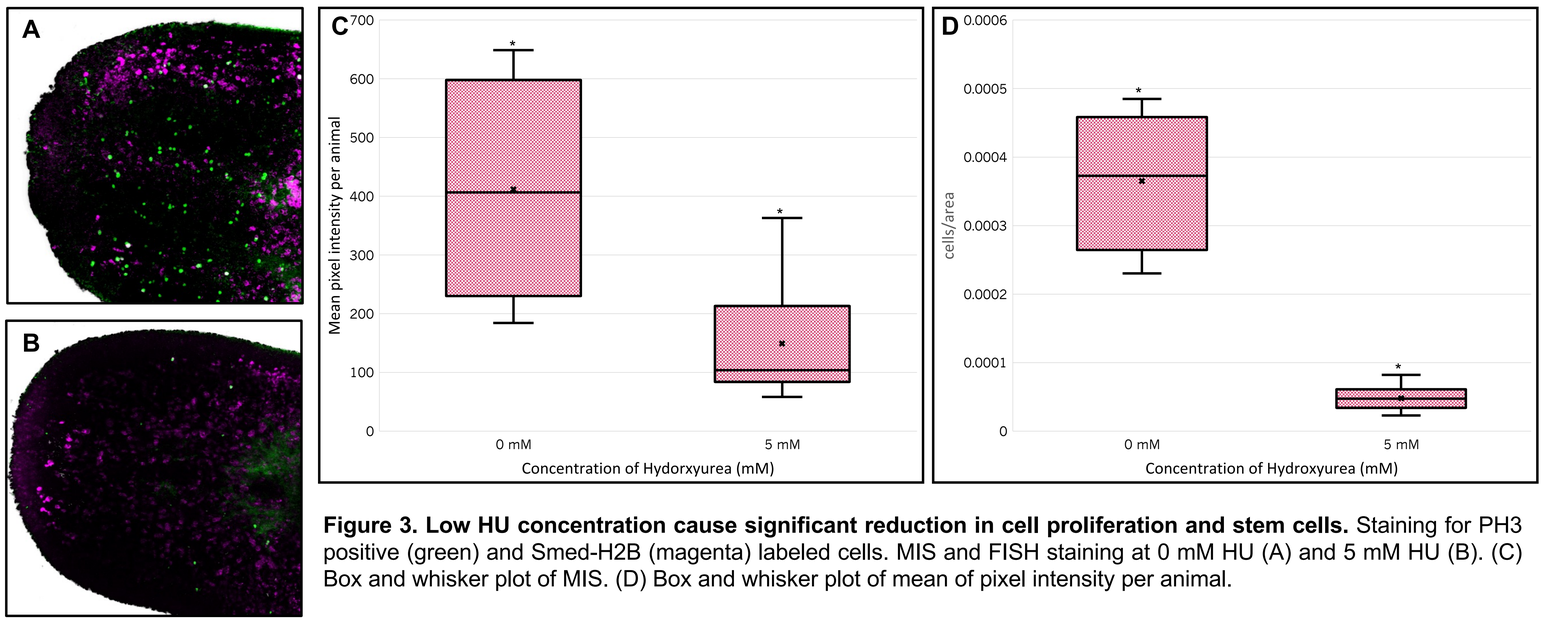

Figure 3. Low HU concentration cause significant reduction in cell proliferation and stem cells. Staining for PH3 positive (green) and Smed-H2B (magenta) labeled cells. MIS and FISH staining at 0 mM HU (A) and 5 mM HU (B). (C) Box and whisker plot of MIS. (D) Box and whisker plot of mean of pixel intensity per animal.

Results and Future Directions

Both the proliferation rate and stem cell pool was dramatically reduced, but not fully eliminated with the treatment of hydroxyurea. Future directions could include longer incubations or higher concentrations of hydroxyurea treatments to completely ablate the stem cell population. An unresolved question we had is whether the remaining stem cells are capable of differentiating. To address this, we have begun performing in situ hybridizations on hydroxyurea treated animals using markers of differentiating cells. To determine whether the stem cells surviving hydroxyurea treatment are viable, we plan to examine whether the stem cells can eventually repopulate the animal by allowing the animals time to recover after hydroxyurea treatment.

References

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3. Agrawal, R. K., et al. (2014). Hydroxyurea in sickle cell disease: drug review. *Indian journal of hematology & blood transfusion: an official journal of Indian Society of Hematology and Blood Transfusion*, 30(2), 91–96. https://doi.org/10.1007/s12288-013-0261-4
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