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EXAMINING UCH-L1 IN MOUSE SPERMATOGONIA

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INTRODUCTION

Spermatogonial stem cells (SSCs) form the foundation of fertility throughout the life of the adult male. However, pathways controlling their self-renewal versus differentiation remain poorly understood. It has previously been established that the deubiquinating enzyme Ubiquitin Carboxyl-Terminal Hydrolase 1 (UCH-L1) is specifically expressed in undifferentiated spermatogonia that contain the SSC population [1]. Based on previous observations that UCH-L1 expression decreases with germ-line differentiation, it is hypothesized that UCH-L1 may play a role in SSC maintenance. The overall goal of the project is to elucidate the role of UCH-L1 in an immortalized type A spermatogonia mouse cell line.

METHODS

This project uses C18-4 cells [2], an immortalized type A mouse spermatogonia cell line, treated with a specific, reversible, active site directed inhibitor of UCH-L1 to examine the growth characteristics of germ cells in the absence of UCH-L1 activity. Cells were treated with 5µM, 10µM and 20µM of UCH-L1 inhibitor compared to DMSO (vehicle) and no treatment control for 24 and 48 hours. Changes in cell number, viability, proliferation (EdU), and apoptosis (TUNEL) were assessed between treatments.

RESULTS

A decreasing trend of cell number and proliferation was observed with increasing UCH-L1 inhibitor concentration. The greatest change in cell number was observed in the 48-hour treatment group with 20µM of UCH-L1 inhibitor ($p=0.0659$) (Figure 1). There was no change observed in TUNEL or cell viability.

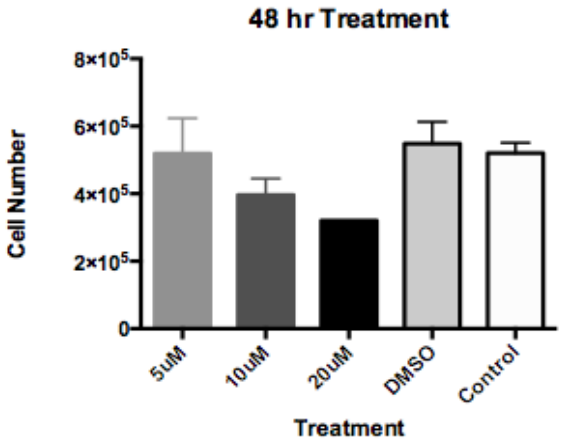

Figure 1. 48-hour treatment of C18-4 cells with UCH-L1 inhibitor. The treatment groups are plotted against the average cell number with standard deviation bars (n=2).

DISCUSSION AND CONCLUSIONS

Results analyzed to date show no significant differences between treatments, which is likely due to low replicate number (n=2). However, a trend of decreasing cell number and proliferation was observed with increasing UCH-L1 inhibitor concentration for both 24 hour and 48-hour treatments. Results of these experiments will help determine the appropriate inhibitor treatment to further examine the mechanistic actions of UCH-L1. Furthermore, elucidating this pathway may lead to new strategies to support expansion of SSCs in vitro.

REFERENCES

1. Luo J, Megee S, Dobrinski I. *J Cell Physiol*. **220**:460-468, 2009.
2. Hofmann M, Braydich-Stolle L, Dettin L, Johnson E, Dym M. *Stem Cells*. **23**:200-210, 2005.