Elinor A. Romaguera
Undergraduate
Louisiana State University, Baton Rouge, Louisiana

Dr. Wu-Min Deng
Tulane University School of Medicine, Department of Biochemistry and Molecular Biology

"RNAi Knockdown Screen Reveals Genes Required for Non-Professional Phagocytosis in *Drosophila* melanogaster"

Introduction: Non-professional phagocytes play a crucial role in preventing diseases like cancer by acquiring phagocytic abilities to engulf apoptotic cells. In *Drosophila melanogaster* oogenesis, these non-professional phagocytes clear egg chambers in response to a halt in oogenesis caused by stressors like starvation, toxins, or temperature shifts. This occurs during the mid-oogenesis checkpoint, in which epithelial follicle cells transform into non-professional phagocytes to engulf and clear dying nurse cells from the egg chamber. With so many factors impacting this process, this study aims to identify genes essential for engulfment. To identify potential genes, we used a previous analysis of a single-cell transcriptomics data set from tumor host flies and chose genes which demonstrated a unique expression in the phagocytic follicle cell in response to a stressor. We expect these genes to show a positive phenotype in which the process of nurse cell engulfment is disrupted.

Methods: 33 genes were chosen for the screen based on results of a prior single cell analysis. RNAi stocks of these genes were obtained from the Bloomington Drosophila Stock Center and were crossed with follicle cell specific driver, Tj-GAL4, UAS-GFP, UAS-GAL80^{ts}. The F1 generations were raised at 18℃ on a cornmeal food media. To obtain a control group with healthy ovaries, flies were kept on a regular diet with added yeast at 29℃ for 3 days. To induce a mid-oogenesis cell death event, flies were kept at 29℃ on a regular diet for 2 days, then moved to a nutritional deprivation environment with 1% agar tubes for 16-24 hours before dissection. Ovaries were dissected in 1X PBS, then placed in 4% formaldehyde for 15 minutes at room temperature on a rocker. After washing with PBT 3 times for 15 minutes each, the DAPI and phalloidin stainers were added for 15 minutes while on a rocker. The stainers were removed and the samples were washed once more in PBT for 1 hour before placing the samples in mounting media. Imaging of the samples was performed on a LSM 900/800 confocal machine for phenotype analysis.

Results: Among the genes screened, we found 6 genes to demonstrate a positive phenotype in which most of the egg chambers showed a significant defect in the nurse cell engulfment process. The genes with phenotypes were *nAChRalpha3*, *Cib*, *Dmtn*, *Six4*, *Ush*, *Hnf4*, and *mys*.

Conclusion: These results give a deeper insight into the process of engulfment by phagocytic follicle cells, and the genes required for it. Because this screen inquires only on which genes produce any phenotype in the egg chambers, further inquiry is required to clarify the implications of each genes' phenotype.