Applying RNAi Screening to Identify Novel Genes that Control Intestinal Stem Cell Proliferation and Gut Regeneration

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Gut epithelium turns over rapidly due to damage from digestion and toxins produced by the enteric microbiota. The rapid turnover process of gut epithelium is tightly regulated. Dysfunction of this regulation promotes either gut atrophy or colorectal cancer. Therefore, developing an understanding of the genetic signaling prompting gut epithelium renewal is critical towards understanding genetic predisposition in colorectal cancer. Current knowledge in regards to how epithelial stress is sensed by enterocytes (ECs) to trigger cytokine production remains poorly understood. In this study, I performed a RNAi-based genetic screening in Drosophila melanogaster gut ECs. I evaluated individual genes' function in sensing stress and producing cytokines through knockdown of candidate genes in ECs and scoring effects on intestinal stem cell (ISC) mitoses. Fly guts were dissected after Pseudomonas entomophila (P.e.) infection and immuno-stained with anti-PH3 antibody. ISC mitoses were scored by PH3+ cells. Of the 300 genes screened, 45 of them (15%) exhibited lower mitotic counts and 85 (28%) exhibited higher mitotic counts compared to wild-type. Lower counts indicate that knockdown of the gene represses ISC mitosis, whereas higher counts indicate increased mitosis. Of the 130 genes that exhibited statistically significant changes, 69 of them are unknown in exact function and are thus unnamed. These genes provide impetus for future research of novel regenerative pathways in the gut. Furthermore, future studies will emphasize on how these genes regulate the damage sensing and cytokine producing capacity of ECs. Through understanding these genes' effects on balancing ISC proliferation and gut epithelium renewal, understanding of colorectal cancer predisposition is advanced.

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