Regulation of cell extrusion by miRNA complexes in *Caenorhabditis elegans*

Vivek Dwivedi, Daniel Denning and Bob Horvitz
HHMI, Dept. Biology, MIT, Cambridge, MA 02139

Cell extrusion, or shedding, is a cell elimination process that occurs in metazoans ranging from sponges to mammals. In humans, approximately 10^{11} cells are extruded from the small intestine epithelium each day. Dysregulation of cell extrusion is deleterious, as inadequate extrusion or excessive extrusion can lead to hyperplasia or a defective epithelial barrier, respectively. Despite the importance of cell extrusion in physiology and relevance to disease, little is known about how extrusion is regulated or what molecules are involved in the process.

We previously identified cells in *C. elegans* that can be eliminated during embryogenesis by either caspase-mediated apoptosis or by cell extrusion and survive when both of these processes are disabled. Such cells survive in mutants with loss-of-function mutations in two genes: *ced-3*, which encodes the caspase required for programmed cell death, and *pig-1*, which encodes a homolog of the protein kinase MELK (Denning *et al.* 2012). For example, the cell ABplpappap survives in *pig-1 ced-3* double mutants and divides to generate an extra excretory cell, resulting in the two excretory cell (Tex) phenotype.

We mutagenized *ced-3* mutants and screened for the Tex phenotype to identify additional cell-extrusion-defective mutants and found that the gene *ain-2* is required for cell extrusion. *ain-2* encodes one of the two *C. elegans* homologs of the mammalian protein GW182, which functions in miRNA-induced silencing complexes (miRISCs). Consistent with the role of a miRNA pathway in cell extrusion, loss-of-function mutations in *alg-2*, which encodes one of the two miRNA-specific Argonaute proteins, also produce the Tex phenotype in a *ced-3* mutant background.

We are currently testing miRNAs that associate with AIN-2 or function during embryogenesis for a role in promoting cell extrusion, by blocking caspase-mediated apoptosis in corresponding mutants and examining for the Tex phenotype. Our preliminary results indicate that the *mir-35* miRNA family might program AIN-2-containing miRISCs to promote cell extrusion in *ced-3* mutants.

Our study reveals that a miRNA pathway is required for cell extrusion and suggests that post-transcriptional regulation of an unknown anti-extrusion factor is a critical step in the mechanism of cell extrusion.

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