296

296. *C. elegans* MUTANTS IN THE *muc-1* GENE PROVIDE A MODEL FOR THE HUMAN LYSOSOMAL STORAGE DISEASE MUCOLIPIDOSIS TYPE IV

Brad Hersh, Erika Hartwieg, Bob Horvitz

HHMI, Dept. Biology, MIT, Cambridge, MA 02139, USA

In a genetic screen seeking suppressors of the *ced-9*(*n1950gf*) allele, which blocks all programmed cell deaths, we identified a single mutation, *n3194*, that resulted in the increased accumulation of refractile corpses and caused recessive maternal-effect lethality. Two additional alleles, *n3264* and *zu223*, were isolated in screens for cell-corpse engulfment-defective mutants and maternal-effect lethal mutants, respectively (Z. Zhou and J. Priess, personal communications).

We found that the predicted gene R13A5.1 rescued the maternal-effect lethal phenotype of *n3194* mutants. R13A5.1 encodes a protein similar to that of the recently identified human mucolipidosis type IV (ML-IV) disease gene, and therefore we have named the gene *muc-1*. ML-IV is a human neurological disorder caused by accumulation of lipid and polysaccharide material in lysosomes. Unlike other lysosomal storage diseases, such as Tay-Sachs disease, in which either catabolic degradative enzymes or activator proteins are defective, ML-IV cells have normal catabolic activity. Instead, ML-IV cells appear to have a defect in the trafficking or sorting of macromolecules, leading to the observed accumulation.

We expressed the human gene (kindly provided by G. Borsani) in *C. elegans* and were able to rescue the maternal-effect lethal phenotype of *muc-1(n3194)*. Using the acidophilic dye Lysotracker Red, we found that *muc-1* mutant embryos accumulate excess lysosomes, just as is observed in the human disease. In addition, electron microscopy indicates that *muc-1* mutant animals contain enlarged vacuoles and lamellar bodies characteristic of the human disease.

Blocking programmed cell death, either by using mutations in the *ced-3* caspase or *ced-4* caspase activator or by overexpressing the p35 viral caspase inhibitor, did not fully block the maternal-effect lethality of *muc-1* mutants but did slightly increase viability. *muc-1* mutants contain an increased number of TUNEL-positive cells, suggesting an increase in programmed cell death, and *ced-3* and *ced-4* mutations block this increase in TUNEL-positive cells. However, as blocking programmed cell death has only a mild effect on the viability of *muc-1* mutants, we suggest that the observed increase in cell death in these mutants is a secondary defect, possibly triggered by the increased accumulation of lysosomal materials. Alternatively, mutations in *muc-1* may activate programmed cell death, or aspects of the cell death process, downstream of *ced-3* and *ced-4*.

A MUC-1::GFP translational fusion capable of rescuing *muc-1(n3194)* was localized to the excretory canal cell and several neurons in the head. Human ML-IV cells have defects in secretion, and the *C. elegans* cells in which MUC-1 is expressed may all have secretory functions for which MUC-1 is required. Proper function of the excretory canal cell is required for viability, so a defect in excretory cell function might be responsible for the lethality of *muc-1* mutants.

We have initiated a screen for suppressors of *muc-1(n3194)* lethality. Genes identified in this screen may define counterparts of the as yet unidentified pathway involved in the human ML-IV disease. We have thus far identified a single recessive suppressor.

We believe that we have developed an excellent *C. elegans* model for the human lysosomal storage disorder mucolipidosis type IV. The etiology of this disease is not yet understood at the molecular level, and we hope that our characterization of the *muc-1* pathway in *C. elegans* will provide a greater understanding of both the normal function of *muc-1* and how the perturbation of its human counterpart results in disease.