The expression of IIR genes correlated with climbing ability in ATM8 and Repo-ATMi flies. (A) Western blot analysis for H2Av-pS137 in adult head extracts from control w1118 (lanes 1 and 2) and ATM8 (lanes 3–8) flies exposed (+) or not exposed (−) to IR. w1118 flies were not separated by the countercurrent assay and ATM8 flies were separated by the counter-current assay into poor, moderate, and good climbers. As a loading control, the same membrane was probed for α-tubulin (bottom). (B–E) Graphed is qPCR analysis depicting the fold change in expression of the indicated genes in ATM8 flies relative to unseparated control w1118 flies or in D and E RepoATMi flies relative to unseparated control Repo-GAL4 flies. Note that the scale is different for each panel. Error bars indicate standard errors of the mean. Asterisks above a bar indicate a significant difference relative to the control and asterisks above a line that spans poor and good climber bars indicate a significant difference between poor and good climber groups. *P, 0.05, **P, 0.01 based on one-way ANOVA analysis.

Another way through which researchers have studied neurodegeneration is to look at cell death in the brain through staining with an antibody to the activated form of Caspase-3 and analyzed by immunofluorescence microscopy. After identifying the correlation between the innate immune response activity and neurodegeneration, relish mutations called RelE20 and RelE38 were examined for lifespan duration and cell death in the brain. These relish mutations did not have relish present in glial cells. In the wild-type control flies, cell death in relish mutations was similarly low with no significant difference to the control flies. Whereas the relish mutations had a significantly reduced cell death in ATM8 and ATM8/+ flies, suggesting that relish is necessary for neurodegeneration.

Figure 2

Relish mutation reduced cell death in the brain of ATM8 flies. (A–C) Graphed is the number of CaspAct-positive cells in the brains of flies of the indicated genotype in a (A) wild-type, (B) ATM8/+, or (C) ATM8 background. Note that the scale is different for each panel. Each circle represents a single brain. Horizontal lines indicate the average. For statistical analysis of A, mutant flies were compared to control w1118 flies. For statistical analysis of B, mutant flies were compared to control ATM8/+ flies. For statistical analysis of C, mutant flies were compared to control ATM8 flies. **P, 0.01, based on one-way ANOVA analysis.

Having identified that relish appears to be necessary for neurodegeneration, to determine whether relish activation is needed for these neurodegenerative outcomes alone, the researchers overexpressed relish in both glial cells and neurons using repo-GAL4 and elav-GAL4, respectively. The CaspAct cell death assay was used to determine the level of neurodegeneration in fly heads once more. The assay revealed that only overexpression of relish in glial cells caused a significant level of neurodegeneration in the fly heads, suggesting that activation of relish in glial cells is sufficient to produce these neurodegenerative outcomes.

Figure 1

Ataxia is a rare human disease which means without coordination. It is caused by the mutation of the A-T gene, which is responsible for the encoding of kinase. Purkinje and granule neurons progressively degenerate in the cerebellum, affecting fingers, arms, legs, speech, hearing, and eye movement, as well as sight. Ataxia can be hereditary or sporadic. There are seven types of ataxia whose symptoms vary but share a commonality of a lack of coordination regarding body movement and a weakened immune system, making the person susceptible to many diseases and early death. The life expectancy of people with ataxia can be as early as their mid-20s or as late as their 60s, though it is uncommon that they live that long. Very little is known about ataxia, and there is no cure for the disease. The treatment surrounding the loss of coordination is rudimentary since it is limited to the use of adaptive aid devices, and multiple types of medication need to be taken to treat each symptom such as slurred speech, depression, tremors, etc., separately. ATM, a fly gene essential in flies and codes for a structurally similar protein to kinase in humans. It plays a critical role in oxidative stress, immunity, DNA damage control, RNA biogenesis, and more. To understand the underlying pathology of the neurodegeneration in A-T, Drosophila melanogaster is used as the model organism for this study. The researchers used temperature-sensitive ATM allele (ATM8) and RNA interference (RNAi) to conditionally inactivate ATM in glial cells. Hence, there were three main experimental groups: homozygous ATM8 mutant (ATM8 mutant (ATM8/+) and repo-ATMi (knock-down). These two types activate the innate immune response in glial cells, causing photoreceptor cell neurodegeneration in fly models of Alzheimer’s disease, suggesting a causative relationship between innate immune response (IMD and Toll Pathways) activation and neurodegeneration.

ATM8 mutant flies, both homozygous and heterozygous, climbed worse than wild-type flies. Repo-ATMi flies followed the same trend. The expression of the innate immune response is correlated to the worse climbing ability in these experimental groups. Since climbing in flies can be used as an indicator of locomotor ability, it is safe to say that worse climbing is an indicator of reduced locomotor ability, because of neurodegeneration. Experimental groups expressed more innate immune response activity and neurodegeneration is to look at cell death in the brain through staining with an antibody to the activated form of Caspase-3 and analyzed by immunofluorescence microscopy. After identifying the correlation between the innate immune response activity and neurodegeneration, relish mutations called RelE20 and RelE38 were examined for lifespan duration and cell death in the brain. These relish mutations did not have relish present in glial cells. In the wild-type control flies, cell death in relish mutations was similarly low with no significant difference to the control flies. Whereas the relish mutations had a significantly reduced cell death in ATM8 and ATM8/+ flies, suggesting that relish is necessary for neurodegeneration.
Overexpression of a constitutively active form of Relish, RelD, in glial cells but not in neurons caused neurodegeneration. (A) Graphed is the number of CaspAct-positive cells in the brains of flies of the indicated genotype. (B–D) Images of paraffin sections of the optic lobe and retina of flies of the indicated genotypes. In B, the optic lobe (OL), lamina (LA), and retina (RE) are labeled. In C and D, open arrowheads indicate vacuolar-like holes in the lamina.

Conclusion

The data gathered suggests that innate immune response activity in ATM gene absent mutant flies is necessary for neurodegeneration, specifically the activation of Relish in glial cells. The IMD pathway has many components to it, including relish. By mutating IMD and Relish, the authors were able to remove IMD as a necessary factor for neurodegeneration. Unfortunately, there are many more factors following IMD in the IMD pathway that may be the source of neurodegeneration. Thus, it is difficult to definitively conclude that relish itself and not a factor following Relish in the IMD pathway affected by the mutation causes neurodegeneration. Mutating other parts of the IMD pathway would be a great future research direction to conduct. Nonetheless, the results imply that stopping relish activation in glial cells in a fly without ATM may stop or reduce neurodegeneration. Does this mean that stopping part of the innate immune response in humans with ataxia may also slow or stop neurodegeneration?

Citations


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