Commentary to: Xie W, Donohue RC, Birchler JA. Quantitatively increased somatic transposition of transposable elements in Drosophila strains compromised for RNAi. PLoS One 2013; 8:e72163; PMID:23940807; http://dx.doi.org/10.1371/journal.pone.0072163

Does ectopic cell death cause somatic mutations in the neighboring cells by activating transposons?

James A Birchler
Division of Biological Sciences; University of Missouri; Columbia, MO USA

Ectopic cell death in Drosophila produces a nonautonomous inhibition of RNA interference (RNAi) in neighboring normal cells. The expression of transposable elements (TE) is increased due to this reduction in the silencing mechanism. New insertions of TE have been documented in mutants for RNAi functions. These observations raise the possibility that persistent environmental insults that produce cell death might increase the frequency of somatic mutations, which might trigger somatic genetic disease.

Homology dependent gene silencing, also known as RNAi, is presumably a mechanism to silence transposable elements as well as viruses in cells of both plants and animals.1 It has also been co-opted to affect the expression of endogenous genes.2 These mechanisms can operate either posttranscriptionally or transcriptionally.1,3 It uses double stranded RNAs that are cleaved by the enzyme Dicer to 21–25 bp lengths that are then used to target other RNAs for destruction. Another system of silencing, the PIWI RNAs or piRNAs are not the product of Dicer but are small RNAs that also target homologous RNAs for elimination.4

Consistent with the hypothesis that homology dependent silencing mechanisms keep transposons in check, evidence that impairing RNAi would increase the expression of transposons was documented. One line of evidence early on was that mutator strains of C. elegans were defective for RNAi functions.5 Subsequently, mutation or silencing of RNAi components was found to increase the expression of transposable elements.6-8 The implication of these studies was that if RNAi components were impaired, then the frequency of new insertions of transposable elements would be found throughout the genome.

Recently, this prediction has been validated in that mutations that block RNAi were found to have new insertion sites for the transposable elements assayed.7 Using mutations in the Dicer 2 and Argonaute 2 genes that, respectively, generate siRNAs from double stranded RNA and that bind to small RNAs, and incorporating them into the line of Drosophila that had been sequenced, these stocks were followed and the distribution of the transposons 297 and Doc were followed by a sensitive fluorescent in situ hybridization technique applied to the larval polytene chromosomes. In the lines homozygous for dcr-2 and ago-2, there was a significant increase in the number of insertions detected for these elements. Many were detected in only some cells of an individual and would therefore be judged to be somatic. Some were found in all cells of an individual and might have represented a germline mobilization.

The inspiration for this test was the finding that ectopic cell death caused an inhibition of RNAi in Drosophila.10 In routine studies of RNAi, a white eye color RNAi construct was found to be reversed for its silencing ability when crossed by a mutation that causes cell death in the eye, namely the classical Bar eye mutant. The white eye color gene normally is responsible for the brick red eye color of a fly. When silenced by RNAi a nearly null phenotype of almost white is found. In
crossing constructs that expressed double stranded RNA for white to the Bar stock, it was found that a red stripe of color was present at the anterior part of the remaining eye tissue. The anterior of the eye is the region adjacent to where cell death has occurred to produce the crescent shaped Bar phenotype.11 When other eye morphology mutations that are known to have cell death in eye tissue were combined with the white RNAi construct, the silencing was reversed in these cases as well.

Then using constructs that fostered cell death in the eye, it was found that these would also reverse RNAi silencing.10 Further, inhibitors of cell death would reverse the RNAi reversal back to the silenced state. And genetic mosaic experiments in which one set of marked cells had cell death occurring among them while adjacent cells were normal revealed that the inhibition of RNAi by cell death could occur in the normal cells. These findings suggested that a signal produced in the dying cells could trigger the inhibition of RNAi in neighboring normal cells.

The generality of this phenomenon was tested by examining flies expressing Green Fluorescent Protein (GFP) together with an RNAi silencing construct in the presence of cell death inducing mutations.10 The GFP expression was restored in this combination. Further, the Head Involution Defective mutation under the control of a low level constitutive promoter exhibited ectopic cell death in most tissues but did not kill the fly. This construct would reverse the silencing of GFP and because cell death was present in many tissues but not lethal, molecular analyses of the phenomenon could be performed.

The results revealed that the ectopic cell death reversed the destruction of the GFP mRNA and caused an accumulation of double stranded RNA homologous to GFP and a reduction of the corresponding siRNA.10 When the expression of transposable elements was examined, there was an increased expression of a similar spectrum of families as had been found to be increased in RNAi mutants. When the total small RNA population was subjected to sequencing and compared with other classes of small RNAs for normalization, it was found that the total amount of siRNA, but probably not the piRNAs, was decreased when there was ectopic cell death.

One possibility for the existence of cell death induced inhibition of RNAi might be that RNAi acts as a first line of defense against viruses.12 If this fails and cell death results from viral proliferation, signaling might occur to neighboring cells to induce processes that modify or bind dsRNA, making them inaccessible for processing to siRNA analogous to the interferon response in mammals.13,14 Thus, the dsRNA cannot enter the RNAi pathway leaving the target mRNA intact. This hypothesis can account for the collective data that indicate a quantitative negative relationship between the cell death signal and the effectiveness of RNAi. Such a second line of defense would be selected if RNAi regularly failed to stop viral infection, which is obviously the case, and would provide a different means of attacking dsRNA. Of course at this stage, other hypotheses to explain the existence of cell death signaling inhibition of RNAi certainly cannot be ruled out and contemplating such other possible mechanisms is worthwhile.

Because cell death inhibition of RNAi elevates TE expression, it is hypothesized to increase TE insertions in adjacent cells. The results found with RNAi mutants indicate that new insertions do in fact occur when RNAi processes are impaired. These findings suggest that chronic diseases or long-term exposure to pathogens, which cause cell death in the stressed tissue, may therefore cause activation of endogenous TEAs in the adjacent somatic cells and may increase the somatic mutation frequency. In humans, somatic mutations have been implicated in diseases, particularly cancer15-17, or if the insertions occur in the germline, the heritable mutation frequency will be increased. The inhibition of RNAi by cell death signaling has yet to be explored in mammals but could have profound implications for initiation of somatic diseases. Recent evidence suggests that RNAi has an anti-viral function in mammals18 and so the same principles might apply.

We therefore suggest that this topic would be a worthwhile endeavor for those studying mammalian species to investigate. If chronic irritants in mammals cause ectopic cell death and in turn increase the expression of transposable elements in normal adjacent cells, then the somatic mutation frequency will likely be increased under these circumstances. Determining whether this is the case or not, and, if true, the mechanism, would have significant implications for the etiology of diseases of somatic origin.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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