Article

15 **Caspase-dependent conversion of Dicer ribonuclease into a death-promoting deoxyribonuclease.**

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Evaluations

Evaluated by Maria Mancini and Nektarios Tavernarakis | Daniel Gallie | Stephen Eacker and Valina Dawson | Zhihua Li and Bino John

This elegant study describes a novel and intriguing role of C. elegans Dicer (DCR-1) as a pro-apoptotic factor in addition to its roles in the processing of small RNAs.

Cleavage of DCR-1 at the carboxyl terminus by the CED-3 caspase abolishes the double strand (ds)RNA dicing activity of DCR-1 but activates a "dormant" DNAse activity that is both necessary and sufficient to promote apoptotic DNA degradation. The findings reported indicate that DCR-1 acts in the same, evolutionarily conserved pathway and likely upstream of the apoptotic nucleases CPS-6, CRN-2, and NUC-1.

Competing interests: None declared

Cite this evaluation

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Cell Biology
07 Dec 2010

Rating 8
Must Read

In this work, the authors show that Dicer-1 (Dcr-1) -- the Dicer ribonuclease in Caenorhabditis elegans responsible for producing the small RNAs that are incorporated into the RNA-induced silencing complex (RISC) to target specific RNA destruction as part of gene silencing -- is a substrate for caspase-mediated cleavage during apoptotic cell death. The cleavage of Dcr-1 by the C. elegans caspase CED-3 inhibits its ribonuclease activity but releases a C-terminal fragment that exhibits DNase activity to produce 3' hydroxyl breaks in the genomic DNA, promoting apoptosis. The work shows for the first time the relationship between the RNA interference (RNAi) and apoptotic pathways in the unexpected role of caspase-cleaved Dcr-1 during the initial degradation of DNA during apoptosis.

A common feature of apoptosis is the activation of nucleases that results in the internucleosomal degradation of genomic DNA to generate fragments of approximately 180 base pairs in length. In mammals, the activation of a nuclease involved in this internucleosomal fragmentation involves the caspase-mediated cleavage of the nuclease inhibitor DFF45, which also serves as a chaperone for its associated nuclease, DFF40. Following cleavage of DFF45, DFF40 is activated to initiate DNA fragmentation by generating 3' hydroxyl DNA breaks. As no candidate for DFF40 or DFF45 is predicted in the C. elegans genome and a reduction in the occurrence of cell death was observed in C. elegans dcr-1 mutants, the authors hypothesized that Dcr-1 could be a direct target of CED-3. RNAi in C. elegans involves the activity of the RNase III-like Dcr-1 to process small RNAs from precursor double-stranded (ds)RNAs that are then delivered to the Argonaute-containing RISC complex to guide sequence-specific silencing of the target mRNA. The authors show that recombinant CED-3 cleaved within the RNase IIIa domain of Dcr-1, generating a C-terminal fragment containing the RNase IIIb domain and a dsRNA binding domain. Not surprisingly, cleavage of the RNase IIIa domain resulted in loss of Dcr-1 RNase activity. The C-terminal fragment, however, exhibited nuclease activity which was not observed with full-length Dcr-1. Truncated Dcr-1 generated 3' hydroxyl DNA breaks, similar to DFF40 in mammalian cells. Mutation of residues required for RNase activity in full-length Dcr-1 resulted in loss of DNase activity as well as the RNase activity. Mutation of the Dcr-1 cleavage site for CED-3 resulted in a mutant protein that could rescue the developmental defects observed in dcr-1 mutants but not the apoptotic phenotype. Expression of the Dcr-1 C-terminal fragment exhibiting DNase activity rescued apoptosis in the dcr-1 mutant but not its developmental defects. An indirect role of the RNAi pathway in apoptosis was ruled out as loss of other components of the RNAi pathway did not produce the dcr-1 mutant phenotype specific to apoptosis. Thus Dcr-1 likely has an RNAi-independent role that is required for internucleosomal degradation during apoptosis. These findings support the conclusion that activation of CED-3 converts Dcr-1 from an RNase

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Rating 10
Exceptional

involved in RNAi to a DNase necessary for the execution phase of apoptosis. Moreover, the authors' results suggest conservation among eukaryotes in the caspase-mediated activation of a DNase to generate 3' hydroxyl DNA breaks that ultimately lead to the internucleosomal fragmentation of genomic DNA. Lastly, the authors' findings raise the question of what other phenotypes attributed to RNAi in Dcr mutants may in fact be due to RNAi-independent mechanisms.

Competing interests: None declared

Cite this evaluation

Despite being the organism wherein programmed cell death was first described, the identity of the enzyme that initiates apoptotic DNA fragmentation had not been identified in Caenorhabditis elegans. This article by Nakagawa and colleagues makes the surprising observation that the small interfering (si)RNA/microRNA producing RNase can be converted into a double-stranded (ds)DNA endonuclease by proteolytic cleavage-initiating apoptotic DNA fragmentation.

Evaluated by:
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Neuroscience
10 May 2010

Rating 8
Must Read

One of the hallmarks of apoptosis is fragmentation of DNA in cells destined for death.
In mammalian cells, DNA fragmentation is initiated by the activation of the endonuclease DFF40 (also known as caspase-activated DNase [CAD]) by the protease caspase 3.
Despite being the organism where apoptosis was first described and genetically dissected, the identity of the DNA fragmentation-initiating nuclease in C. elegans has remained a mystery.
The study by Nakagawa et al. identifies a proteolytically cleaved fragment of the RNase III family member Dicer as the missing caspase-activated nuclease in C. elegans.
Using a convincing series of genetic and biochemical experiments, the authors demonstrate that CED3 (the caspase 3 homolog in C. elegans) cleaves Dicer in the first of two RNase III domains, leading to the activation of an endonuclease which results in TUNEL reactivity in apoptotic cells in vivo.
This fascinating report illustrates several important considerations beyond the important step of identifying the DNA fragmentation initiator.
Despite the deep conservation of the caspases and their role in apoptosis, the effector molecules differ between species.
This illustrates how even deeply conserved processes have important species-specific differences.
In a similar vein, the differences in Dicer function between C. elegans and mammals are also highlighted in this study.
While Dicer is essential for life in mammals {1}, C. elegans tolerates the complete loss of Dicer function {2}.
In contrast, Dicer is required (in part) for programmed cell death in C. elegans.
Though it seems unlikely that Dicer will play a significant role in the execution of cell death in mammals, it remains to be seen if similar proteolytic events modify Dicer function outside of C. elegans.

References: {1} Bernstein et al. Nat Genet 2003, 35:215-7 [PMID:14528307]. {2} Grishok et al. Cell 2001, 106:23-34 [PMID:11461699].

Competing interests: None declared

Cite this evaluation

This article reports a surprising cellular switch that can convert Dicer from a ribonuclease involved in the RNAi pathway to a deoxyribonuclease involved in apoptosis.

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Genomics & Genetics
01 Apr 2010

Rating 6
Recommended

Dicer is a well-known ribonuclease central to the miRNA/siRNA processing pathway. In this study, the authors found that the Caenorhabditis elegans Dicer gene (dcr-1) plays a previously uncharacterised role in apoptotic cell death. By knockdown and genetic assays, they found that DCR-1, but not other proteins in the RNAi pathway, is essential to apoptotic DNA degradation and programmed cell death. This unexpected role of Dicer requires its cleavage by the C. elegans caspase CED-3, which converts Dicer from a ribonuclease that cuts double-stranded RNA to produce miRNA/siRNA, to a deoxyribonuclease that cuts chromosomal DNA to promote cell death. Interestingly, the same set of conserved acidic residues is important for both the ribonuclease and deoxyribonuclease activities of Dicer, suggesting a similar catalytic mechanism for both activities. This surprising finding has an impact on both cellular and structural biology. It is of interest to see 1) if this interplay between the small RNA processing pathway and the apoptosis pathway is conserved in mammals and 2) if this cleavage-mediated substrate change can be found in other nucleases.