

AN ALTERNATIVE, NON-APOPTOTIC FORM OF PROGRAMMED CELL DEATH

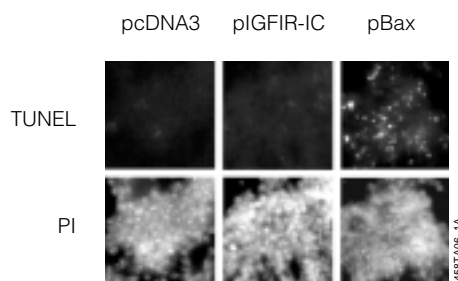
Sperandio, S. *et al.* (2000) *Proc. Natl. Acad. Sci. USA* **20**, 14376–14381.

The authors use Promega's Apoptosis Detection System, Fluorescein (Cat.# G3250) to investigate events associated with IGFIR-IC-induced cell death.

Traditional thought about cell death focuses on two distinct types of events. Apoptosis is characterized by distinct morphological characteristics and specific genetic events; necrosis is described by different morphological characteristics and a lack of genetic control of the death process. However some forms of cell death, including oncosis and some developmental and neurodegenerative cell deaths, do not fit morphological descriptions of necrosis or apoptosis. The biochemical mechanisms by which these alternative cell deaths occur are unknown. In this study, the authors describe a form of cell death that requires gene expression but does not conform to morphological and biochemical criteria for apoptosis.

In 293T cells and Apaf-1-null mouse embryonic fibroblasts transfected with the human insulin-like growth factor I receptor β subunit intracellular domain (IGFIR-IC), expression of IGFIR-IC induces a form of cell death that lacks key morphological features of apoptosis. Actinomycin D and cycloheximide inhibit this cell death in transfected 293T cells, indicating that it requires transcription and translation, distinguishing it from necrosis. This alternative programmed cell death, named paraptosis, does not involve nuclear fragmentation, chromatin condensation, or apoptotic body formation like traditional apoptosis but instead displays cytoplasmic vacuolation and mitochondrial swelling similar to necrosis. The nuclear events of paraptosis also differ from apoptosis. In contrast to control cells transfected with a Bax expression construct, IGFIR-IC-transfected cells show no internucleosomal DNA fragmentation and do not label in the TUNEL assay using Promega's Apoptosis Detection System, Fluorescein^(a) (Cat.# G3250).

Apoptotic inhibition by caspase inhibitors (Z-VAD-FMK, BAF, p35, and X-chromosome-linked inhibitor of apoptosis) and Bcl-xL does not block paraptosis. However, a catalytic mutant of caspase-9 zymogen inhibits



IGFIR-IC-induced cell death is nonapoptotic. TUNEL staining of cells transfected with control vector (pcDNA3), Bax (pBax), or pIGFIR-IC, 48 hours after transfection. The upper panel shows FITC staining from the TUNEL assay; the lower panel shows the corresponding propidium iodide staining. The pIGFIR-IC-induced cell death is not apoptotic as indicated by TUNEL analysis. Figure reprinted with permission of Dr. D. Bredesen, Buck Institute of Age Research and the National Academy of Sciences.

paraptosis, while similar mutations of other caspases do not. Caspase-9 appears to mediate IGFIR-IC-induced cell death; coimmunoprecipitation assays of caspase-9 and IGFIR suggest that IGFIR recruits caspase-9 and activates the cell death process. Proapoptotic and preparapoptotic activities of caspase-9 can be distinguished by a lack of response to caspase inhibitors in paraptosis.

These results have potential implications for morphologically similar cell deaths observed in development and neurodegeneration and raise questions about the evolutionary relationship between apoptosis and other programmed cell deaths.

Review by Rebecca Sanders, B.S. Agnes Scott College, Decatur, GA.

Ordering Information

Product	Size	Cat. #
Apoptosis Detection System, Fluorescein ^(a)	60 reactions	G3250
Caspase Inhibitor, Z-VAD-FMK	50 μ l	G7231
	125 μ l	G7232

For Laboratory Use.

^(a) The Fluorescein-12-dUTP component is manufactured for Promega Corporation by NEN® Life Science Products under U.S. Pat. Nos. 5,047,519 and 5,151,507. NEN is a registered trademark of du Pont NEN Research Products.