Promega's Anti-PARP p85 Fragment Antibody for Apoptosis Detection

This article highlights peer-reviewed literature that demonstrates the use of Anti-PARP p85 Fragment pAb for detection of apoptosis by immunohistochemical and immunocytochemical analyses.

Apoptosis has become one of the most widely researched cell processes in biology, and approximately 20 apoptosis-related papers were published daily in 2000 (1). Induction of apoptosis often indicates toxicity, and therefore detection of apoptosis is important for drug discovery research. Identifying cells as clearly having adopted an apoptotic cell fate often requires more than one method for determining apoptosis. TUNEL assays are a common method for assessing apoptosis, but at least one study has shown that TUNEL analysis may be inadequate when evaluating tissue samples from various disease states (2).

PARP, poly(ADP-ribose) polymerase, is a nuclear DNA-binding protein that detects DNA strand breaks and functions in base excision repair (3). Once PARP is cleaved, it no longer supports the enzymatic DNA repair function, and there is some evidence that cleaved PARP may inhibit access to DNA by other repair enzymes (4). Although PARP is not absolutely required for apoptosis to proceed, the cleavage of PARP may contribute to the commitment to apoptosis (3,4).

PARP cleavage is detectable earlier than other events associated with apoptosis.

Anti-PARP p85 Fragment pAb[a] is a polyclonal antibody directed against the 85kDa caspase-cleaved fragment (p85) of human PARP. Anti-PARP p85 Fragment pAb specifically detects the 85kDa (apparent molecular weight) fragment of PARP and does not detect the 116kDa intact PARP molecule. The specificity of the antibody for cleaved PARP makes it an excellent tool for detecting apoptosis. Anti-PARP p85 Fragment pAb can be used for immunocytochemical or immunohistochemical studies. Additionally the antibody can be used for Western blot analysis (5). The cleavage of PARP is a fairly early event in apoptosis, and several studies indicate PARP cleavage is detectable earlier than other events associated with apoptosis such as DNA fragmentation (6).

Acetaminophen-Induced Apoptosis in Liver

Zhang *et al.* (7) used Promega's Anti-PARP p85 Fragment pAb as a marker for apoptosis in mouse liver. In their study, they present the first report of a role for FAS in acetaminophen (AAP)-mediated liver toxicity, showing that FAS expression increases in response to AAP and that AAP-

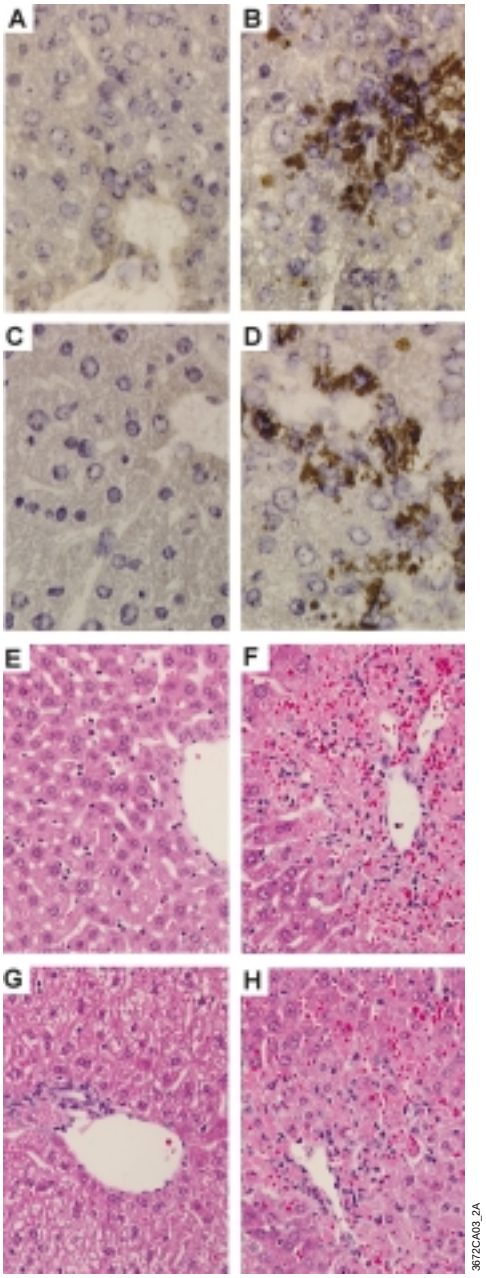

Figure 1. Effect of antisense oligonucleotide-mediated Fas inhibition in mouse liver on AAP-induced hepatocyte apoptosis. Mice were treated with a 2´-MOE-modified antisense oligonucleotide (**Panels C and G**) or an eight base mismatch control oliognucleotide (**Panels D and H**) (40mg/kg daily × 4, intraperitoneal). Four hours after the last dose, mice were given AAP (300mg/kg, intraperitoneal). Animals were killed 24 hours after dosing with AAP, and liver sections were processed for immunohistochemical analysis. Sections were stained for either apoptosis with Anti-PARP p85 Fragment pAb (**Panels A–D**) or with Hematoxylin and Eosin (**Panels E–H**). Reprinted from Zhan, *et al.* (2000) *Nature Biotechnology* **18**, 862–867, with the kind permission of Dr. Hong Zhang and *Nature* Publishing.

mediated liver damage is reduced when FAS expression is reduced by the treatment with a 2′-O-(2-methoxy)ethyl (2′-MOE) modified antisense oligonucleotide. Figure 1 shows the effect of antisense oligonucleotide inhibition of FAS expression on AAP-induced hepatocyte apoptosis.

Morphology Changes in Apoptotic HeLa Cells

Lane *et al.* (8) report that the Golgi complex fragments into small tubules and vesicles during apoptosis and that this fragmentation is caspase-dependent. In apoptotic HeLa cells, indicated by positive Anti-PARP p85 Fragment pAb staining, the Golgi apparatus was fragmented. When cells were pretreated with a caspase inhibitor, Golgi fragmentation was not observed.

Initial studies of GRASP65, a Golgi stacking protein, suggested that it might be involved in the apoptosis-mediated fragmentation. GRASP65 immunostaining was lost in apoptotic NRK cells. Immunoblots reveal that GRASP65 is cleaved in NRK cells treated with the apoptosis inducer, staurosporine, but not in untreated NRK cells or NRK cells treated with staurosporine and a caspase inhibitor. Additionally, Anti-PARP p85 Fragment-positive cells do not react with a GRASP65 monoclonal antibody (Figure 2). The process of Golgi fragmentation was correlated with the progression of apoptosis in HeLa cells (Figure 3).

These two references demonstrate the utility of the Anti-PARP p85 Fragment pAb for detecting apoptosis. Cleaved PARP staining provides a rapid assessment of apoptosis that complements other detection methods such as TUNEL staining.

References

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Protocol

Anti-PARP p85 Fragment pAb Technical Bulletin #TB273
www.promega.com/tbs/tb273/tb273.html

Ordering Information

Product	Size	Cat.#
Anti-PARP p85 Fragment pAb	50µl	G7341

[a] U.S. Pat. No. 6,350,452 has been issued to Promega Corporation for apoptosis marker antibodies and methods of use.

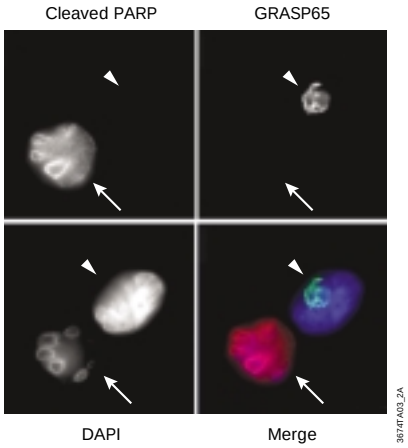

Figure 2. GRASP65 is a substrate for caspase-mediated proteolysis during apoptosis. NRK cells treated with 1µM staurosporine for 4 hours were processed for immunofluorescence microscopy. **Panel A.** Cells were labeled with Anti-PARP p85 Fragment pAb (Cat.# G7341), a monoclonal antibody to GRASP65 (**Panel B**), and DAPI (**Panel C**). **Panel D** shows a merged figure showing cleaved PARP (red), GRASP65 (green), and DAPI (blue). Image reprinted with the kind permission of Dr. Martin Lowe and the *Journal of Cell Biology* (2002) **156**, 495–509, by copyright permission of The Rockefeller University Press.

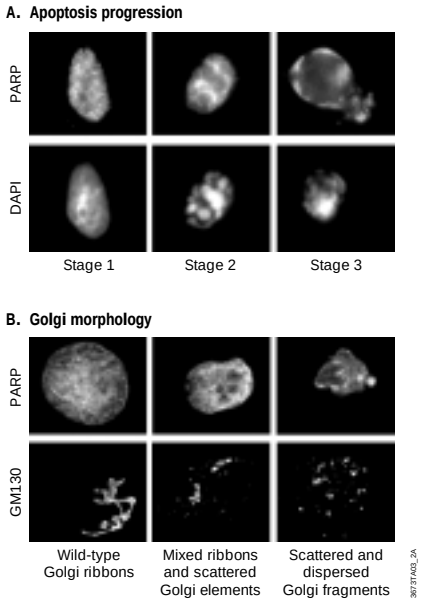

Figure 3. The progress of Golgi fragmentation during apoptosis. Staurosporine-treated HeLa cells were fixed and stained with a monoclonal antibody against GM130, a Golgi marker and with Anti-PARP p85 Fragment pAb. Cells were characterized by for apoptotic progression (**Panel A**). The condition of the Golgi apparatus was then assessed in apoptotic cells (**Panel B**). Image reprinted with permission of Dr. Martin Lowe and the *Journal of Cell Biology* (2002) **156**, 495–509, by copyright permission of The Rockefeller University Press.