Polycystin-1, the Gene Product of PKD1, Induces Resistance to Apoptosis and Spontaneous Tubulogenesis in MDCK Cells

Boletta, A. *et al.* (2000) *Mol. Cell* **6**, 1267–1273.

The authors use Promega's Apoptosis Detection System, Fluorescein (Cat.# G3250) to examine the effect of expression of PKD 1+ in MDCK cells on the induction of apoptosis.

Autosomal dominant polycystic kidney disease is a common genetic disease in which renal cysts form and increase in size throughout a person's lifetime, resulting in end-stage renal disease in 50% of patients by the age of 60. Approximately 85% of all cases are caused by mutations in the *PKD1* gene encoding polycystin-1, a possible membrane-bound receptor protein. *PKD1* mutations are known to interfere with renal tubular differentiation, cell proliferation and apoptosis.

Boletta *et al.* studied the function of *PKD1* by transforming a Madin Darby canine kidney (MDCK) cell line with a full-length human *PKD1* cDNA clone. MDCK cell lines are widely used in renal tubulogenesis studies. These cells spontaneously form cysts when cultured in three dimensional collagen gels but undergo tubulogenesis when treated with hepatocyte growth factor (HGF).

The growth rate and morphogenic properties of control and *PKD1*-transformed cell lines were compared in 3D collagen gel cultures without HGF. The growth rate of the control cells was twice that of *PKD1* cells.

After 1 week, control cell lines had formed cystic structures, while the *PKD1* cell lines had formed no visible multicellular structures. After 3 weeks, 14/14 control cell lines primarily formed cysts, while 11/11 of the *PKD1* cell lines formed mostly tubular-like structures. The ratio of tubular versus cystic structures formed by a cell line correlated to the level of expression of the *PKD1* transgene in that cell line.

Apoptosis has been implicated in cyst formation by MDCK cells in 3D collagen gels. To test the effect of *PKD1* expression on apoptosis, apoptosis was induced in PKD1 and control cells by serum starvation. A cell count after 72 hours showed a decrease in control cells. TUNEL assays using Promega's Apoptosis Detection System, Fluorescein[(a)] (Cat.# G3250) demonstrated that a majority of the control cells were apoptotic, while only 5% of the *PKD1* cells underwent apoptosis (Figure 1).

Using a simple cell culture system, the authors have shown that the *PKD1* gene product affects cell morphology, growth rate and apoptosis. They suggest that polycystin-1 may decrease proliferation of epithelial cells in developing tissues, allowing cells to enter a differentiation pathway resulting in tubule formation instead of apoptosis.

Ordering Information

Product	Size	Cat.#
Apoptosis Detection System, Fluorescein	60 reactions	G3250

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.

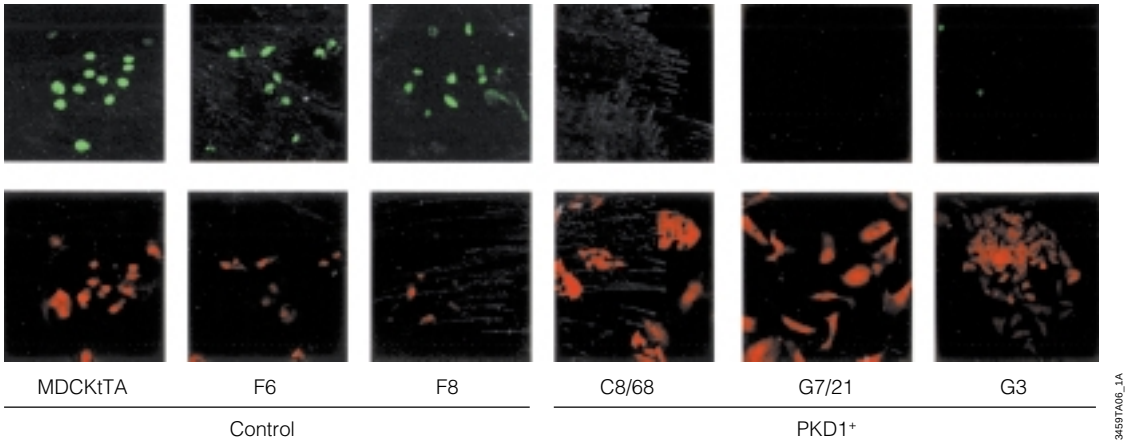

Figure 1. TUNEL assay of control and PKD1+ cell lines 72 hours after serum starvation. Fluorescein-12-dUTP(a)-labeled DNA (green) was visualized by confocal microscopy (60X), and total nuclei were counterstained using propidium iodide (red). Figure reprinted with permission of Dr. A. Boletta (Johns Hopkins University School of Medicine) and Cell Press.