Distinguishing apoptosis from necrosis by using time-lapse microscopy

Time-lapse microscopy is the method of choice for the dynamic monitoring of individual cells for the morphological changes that occur during cell death, and for appreciating the differences between the apoptotic and necrotic types of cell death. In this setup as an example we used L929sA fibrosarcoma cell line, in which apoptotic as well as necrotic cell death can be induced. Stimulation of TNFR1 in L929sA cells leads to necrotic cell death, whereas in L929sA cells transfected with human Fas (L929sAhFas), the use of agonistic anti-Fas antibodies leads to clustering of Fas and induction of the apoptotic cell death pathway (Vercammen et al., 1997).

Onset of apoptosis is characterized by rounding up of the cell, blebbing, and formation of apoptotic bodies, and it culminates with formation of balloon-like structure indicating the loss of plasma membrane integrity and development of secondary necrosis. In contrast to apoptosis, necrosis is characterized by cellular swelling and formation of balloon-like structures. These distinct morphological features of apoptotic versus necrotic cell death could be appreciated on time-lapse movies (L929sAhFas cells, apoptosis morphology.avi; necrosis morphology.avi).

Secondary necrosis can be distinguished from primary necrosis by staining the nucleus with fluorescent probes such as PI. Secondary necrotic cells had passed an apoptotic cell death stage and their nuclei are fragmented or condensed; PI homogenously stains the nucleic acids content due its binding to DNA by intercalating between the bases. Primary necrotic cells have uncondensed nuclei with prominent nucleoli. The distinct pattern of staining with PI of apoptotic and necrotic L929sAhFas cells could be appreciated on time-lapse movies (apoptosis PI.avi and necrosis PI.avi).

The dynamics and interrelation between phosphatidylserine (PS) exposure and PI permeability during apoptosis and necrosis in L929sAhFas cells could be appreciated on time-lapse movies (apoptosis PS PI.avi and necrosis PS PI.avi).

A more detailed information about the possible methods, which could be used to distinguish apoptosis from necrosis can be found in the article: DV Krysko, T Vanden Berghe, E Parthoens, K D’Herde and P Vandenabeele Methods for distinguishing apoptotic from necrotic cells and measuring their clearance. Methods in Enzymology. In press, 2008.