[Frontiers in Bioscience 5, f1-2, September 1, 2000]

APOPTOSIS – WHEN THE CELLS BEGIN TO DANCE

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1. ABSTRACT

We present a 24-hour time-lapse videosequence of *in vitro* behavior of Hep-2 cells treated with 10 μg/ml Etoposide, a topoisomerase II inhibitor. The cell behavior was recorded by a Mitsubishi video recorder, HS-S5600. In the presented sequence, we show the typical cell rounding accompanied by formation of numerous pseudopodia and rapid rhythmical contractions, so called membrane blebbing known as "dance of death".

2. INTRODUCTION

There are many methods for defining cell death. The microscopic examination of cell morphology still remains standard to all cell death investigations (1-3). On the other hand, most methods, including microscopy, grasp just one precise moment of cell death, and not its dynamics. Therefore, some authors have begun to use time-lapse videomicroscopy for establishing cell death (4-6). Here, we present the recorded *in vitro* behavior of Hep-2 cells undergoing apoptosis induced by Etoposide, a topoisomerase II inhibitor.

3. MATERIALS AND METHOD

Etoposide (Vepesid inj) was purchased from Bristol-Myers Squibb (Regensburg, Germany). The tested concentration was prepared by diluting the original solution in a medium directly before each experiment.

Human laryngeal cell line Hep2 (EATCC, No. 86030501, Porton Down, United Kingdom) was maintained as stationary monolayer in plastic tissue-culture dishes Nunclon (Roskilde, Denmark). Cells were grown in Dulbecco's modified Eagle's medium Sevapharm (Prague, Czech Republic), supplemented with 10% bovine serum Bioveta (Ivanovice, Czech Republic), 100 U/ml penicillin, and 100 μ g/ml streptomycin. The medium was changed every third day, and cells were passaged using 0.25% trypsin.

After 24 hours of cultivation, the standard medium was replaced with a medium containing 10 μg/ml Etoposide. The culture flask was left in an incubator for 20 minutes and then transferred to a 37 °C heated chamber where all recordings were performed. Cells were examined during 24 hours, using an inverted microscope (Olympus IMT-2) equipped with a long-working-distance condenser, and a 20 x phase contrast lens. For time-lapse recording, the microscope was equipped with a Mitsubishi CCD-100E camera and connected to a Mitsubishi video recorder HS-S5600. The recording was performed in a 480 mode, with a slowing factor 160 and it continued for 48 hours, with a subsequent film analysis. The chosen sequences have been spliced and converted to the digitized form by the software LUCIA DI Image Analysis System LIM (Prague, Czech Republic) and adjusted to the MPEG format by Ulead Video Studio v.4, Xing MPEG Encoder v.2.1.

4. RESULTS AND DISCUSSION

During this study, we recorded over 20 video-sequences, with the control and treated cells in parallel. The presented typical sequence (figure 1) shows cell rounding (2-6 hours after beginning of the treatment) accompanied by formation of numerous pseudopodia. Also shown are rhythmical contractions, so called membrane blebbing (the early stage – 8 hours after beginning of the treatment, the late stage – 16 hours after beginning of the treatment). In the shown sequence, the apoptosis marked by blebbing occurs individually. The entire process lasts up to 12 hours, and after 24 hours, cells remain motionless and shrunken.

Many methods should be combined to exclude the possibility of false results while studying apoptosis (7). Among the established procedures, time lapse videomicroscopy has become an accepted modality for validating apoptosis (7). Videomicroscopy allows recording of the cell behavior during the entire course of apoptosis, and particularly offers the opportunity to view and record

Figure 1. The video sequence of time lapse videomicroscopy showing behavior of Hep-2 cells treated with Etoposide for 24 hours.

the violent movement of the cytoplasmic membrane, a phenomenon known as membrane blebbing (7-9). This membrane blebbing is very impressive and has been nicknamed the "dance of death". Here, we showed the cell behavior during apoptosis induced by Etoposide, a topoisomerase II inhibitor. Our results concur with those reported previously (3-6, 8,9).

5. ACKNOWLEDGMENTS

This work was supported by ministry of education czech republic research Project FD MSM 111500002 pathogenesis, diagnostics and therapy of malignant diseases and research project FD MSM 111500004 the development of software For processing of medical information and biosignals supporting improvement of and for improvement of quality of education.

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Key Words: Apoptosis, Blebbing, Detection, *In vitro*, Time-lapse Videomicroscopy

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