[Frontiers in Bioscience 7, f8-9, January 1, 2002]

3D-COMPUTER BASED RECONSTRUCTIONS OF APOPTOTIC NUCLEI

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1. ABSTRACT

We present a 3D-model of apoptotic nuclei of HL-60 cells treated with 10 μg/ml Etoposide (topoisomerase II inhibitor) for 24 hours. The static model was generated from a series of optical sections obtained through a confocal microscope by freeware and shareware graphical programs available in the Internet. Its animation was done by 3D Studio Max. We demonstrate the appearance of typical fragmentation and condensation of chromatin accompanied by its aggregation to the inner side of the nuclear membrane.

2. INTRODUCTION

Light and electron microscopy are routinely used for identification of fragmented and condensed chromatin in apoptotic cells /1, 2/. However, they generate only a 2D-image, which is not often sufficient for understanding of the spatial relationships inside the nuclei. To challenge this situation, we decided to create 3D-models of apoptotic nuclei using the series of optical sections obtained by a confocal microscope with subsequent computer-based reconstruction and animation. We present a 3D-model of apoptotic nuclei of HL-60 cells treated with 10 μg/ml Etoposide, a topoisomerase II inhibitor.

3. MATERIALS AND METHOD

Etoposide (Vepesid inj.) was purchased from Bristol-Myers Squibb (United Kingdom). The tested concentration was prepared by diluting the original solution in a medium directly before each experiment. All other chemicals were obtained from Sigma-Aldrich (U.S.A.).

Human promyelocytic cell line HL-60 (ECACC, No. 98070106, Porton Down, United Kingdom) was maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were passaged three times a week.

Twenty-four hours old culture was treated with 10 μg/ml Etoposide for 24 hours. Following the treatment, cells were fixed in 2% paraformaldehyde and stained with propidium iodide at concentration of 1 μg/ml for 10 minutes. The stained nuclei were optically sectioned by a confocal microscope LSM V (Zeiss, Germany). Individual series contained 20 sections, each 0.5 μm thick, which were stored in digital format TIFF. The resulting images were computer processed, using the freeware programs available at the Internet address: http://synapses.bu.edu/tools/index.stm. The following programs were employed: CONVERT (conversion of TIFF into BMP format), IGL TRACE (manual bordering of the edges, preparation of the wire model in the format WRML 1.0), and 3D-CROSSROADS (conversion of the format WRML into 3-D format). The static 3-D models were finalized by graphic studio RHINOCEROS (v.1.1) available as a shareware program at http://www.rhino3d.com/. The animated 3-D models were prepared by 3D Studio Max v. 3.1.

4. RESULTS AND DISCUSSION

Fluorescent images obtained through confocal microscopy show chromatin fragmentation typical of apoptosis (figure 1). The process of 3-D reconstruction by bordering the nucleus and chromatin fragments as well as preparation of the wire model are depicted in figures 2, 3.

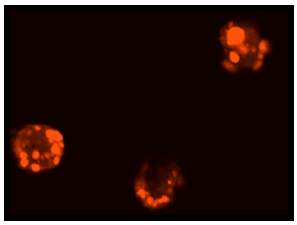

Figure 1. The typical chromatin fragmentation in HL-60 cells treated with Etoposide during 24 hours.

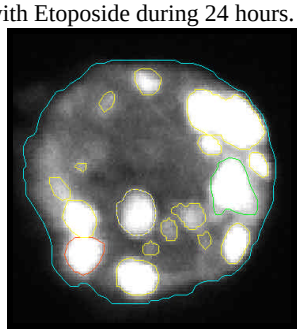

Figure 2. The process of nucleus and individual chromatin fragments bordering using the program *IGL TRACE*.

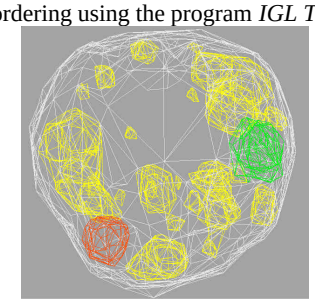

Figure 3. The wire model of the apoptotic chromatin as prepared by the program *IGL TRACE*.

Figure 4. The finalized 3-D model of the apoptotic chromatin as prepared by graphic studio *RHINOCEROS (v.1.1).*

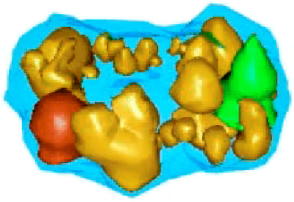

Figure 5. The animated 3-D model of the apoptotic chromatin as prepared by 3D Studio Max v. 3.1.

The finalized model (figure 4) shows the position and spatial organization of individual chromatin fragments 24 hours after beginning of the treatment, with its animated form presented in figure 5.

During this study, we generated over 10 3-D models, with the control and treated cells in parallel. We found that the source images obtained through confocal microscopy can be used for 3-D modeling, however, in some cases "the halo effect" of the individual fluorescent objects produces difficulties in the process of bordering. Thus it seems that images obtained through electron microscopy would be more suitable for this type of modeling (3, 4).

In our opinion, this type of computer-based 3-D reconstructions is not destined to become a routine method for studying morphological changes during apoptosis, in particular due to its laborious and time-consuming preparation. On the other hand, once such a model is prepared it may be regarded as useful supplement to the apoptotic studies as much as it is useful in general investigations of chromatin structure (5).

5. ACKNOWLEDGMENTS

We would like to express our gratitude to professor Josef Špacek who introduced us to the basic principles of computer-based 3-D reconstruction software. This work was supported by Ministry of Education Czech Republic Research Project FD MSM 111500002 Research Project FD MSM 111500004.

6. REFERENCES

1. Wilson J.W, and Ch. S. Potten: Morphological recognition of apoptotic cells. In: Apoptosis. A practical approach. Ed: Studzinski G.P, Oxford University Press, NC, 15-25 (1999)
2. Gorman A, J. Mccarthy, D. Finucane, W. Reville & T. Cotter: Morphological assessment of apoptosis. In: Techniques in Apoptosis. A user's guide. Eds: Cotter T.G, Martin S.J, Portland Press, London, UK, 1-20 (1996)
3. Ventura R & K.M. Harris: Three-dimensional relationships between hippocampal synapses and astrocytes. *J Neuroscience* 19, 6897-6906 (1999)
4. Lieberman A.R & J. Spacek: Filamentous contacts: the ultrastructure and three-dimensional organization of specialized non-synaptic interneuronal appositions in thalamic relay nuclei. *Cell Tiss. Res*. 288, 43-57 (1997)
5. Montag M, H. Spring, M.F. Trendelenburg & A. Kriete: Methodical aspects of 3-D reconstruction of chromatin architecture in mouse trophoblast giant nuclei. *J Microsc* 158. 225-233 (1990)

Key Words: Apoptosis, Nucleus, *In vitro*, 3-D reconstruction, Computer-assisted, Image processing, Microscopy, Confocal microscopy

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