

# Delayed Reproductive Death and ROS Levels in the Progeny of Irradiated Melanoma Cells

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Z. Naturforsch. **59c**, 297–301 (2004); received October 13/November 7, 2003

The cell death and survival of proliferating (clonogenic) cells were investigated in two human melanoma cell lines to assess the optimal conditions for preparation of apoptotic bodies from melanoma cells. After 50 J/m<sup>2</sup> UVB+UVC the maximal levels of apoptotic cells assayed by Trypan blue staining, nucleosomal DNA fragmentation, MTT, and TUNEL tests were observed within 2–3 d of radiation. In 100 Gy gamma-irradiated cultures these apoptosis indicators were delayed for up to 3 weeks. In addition, clonogenic cells were observed only in exponentially growing cultures irradiated with UV at high cell density but not in gamma-irradiated cultures. The response of melanoma cultures after high UV radiation doses contrasted to the response in lethally gamma-irradiated cultures. UV-irradiated melanoma cultures were recovered within two weeks. Most of the clonogenic cells in the recovered colonies contained micronuclei. ROS levels determined by DCF fluorescence and a modified MTT test were also normalized obviously due to the extensive antioxidant defense system of melanoma cells.

UV radiation of tumor cells might be the preferential method for preparation of apoptotic bodies. The presence of clonogenic cells in the suspension of apoptotic bodies from melanoma cells used for pulsing of dendritic cells with tumor antigens might compromise this protocol for preparation of cell vaccines.

*Key words:* Radiation, Clonogenic Cells, ROS