

# Evaluation of Delayed Apoptotic Response in Lethally Irradiated Human Melanoma Cell Lines

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To assess the lethal doses of gamma radiation and corresponding apoptotic response in new established human melanoma cell lines we exposed exponentially growing cultures to 8–100 Gy gamma radiation. The apoptosis and cell survival were determined by trypan blue exclusion, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) reaction, agarose gel electrophoresis, colony forming assay, and long-term survival assay. The maximal DNA fragmentation 3 days after irradiation was observed in cultures irradiated with 20 Gy (36.9% TUNEL positive cells). The cultures irradiated with 50 and 100 Gy contained 18.7% and 16.4% TUNEL positive cells, respectively. Cultures exposed to 8 and 20 Gy gamma radiation recovered by week 3–4. Lethally irradiated (50 and 100 Gy) cultures which contained less apoptotic cells by day 3 died by week 5. A detectable increase in melanoma cell pigmentation after irradiation was also observed. The survival of human melanoma cell cultures after exposure to gamma radiation does not correlate with the level of apoptotic cells by day 3. At high radiation doses (> 50 Gy) when the radiation induced cell pigmentation is not inhibited the processes of apoptotic DNA fragmentation might be preferentially inactivated.