Radiation-induced apoptosis and its relationship to loss of clonogenic survival

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Ionizing radiation can be an effective inducer of apoptosis and studies of many aspects of the pathways and mechanisms involved in this apoptosis induction have been published. This review stresses two aspects: the relationship between apoptosis and loss of clonogenic ability in irradiated cells and the time course for the appearance of apoptosis after radiation exposure. Although it was initially assumed that apoptosis occurred relatively quickly (within hours) after irradiation, evidence is presented and discussed here showing that apoptosis can occur at long times after irradiation (out to 20 days) in some cell types. This late, or delayed, apoptosis occurs after the cells have divided once or several times. The impact of delayed apoptosis on loss of clonogenicity after irradiation remains unclear. It seems likely that in some cell types, e.g., fibroblasts, the occurrence of late apoptosis is minimal and may have little impact on long term cell survival of the population, but in at least one instance, with a cell line of hematopoietic origin, it appears that late apoptosis can account for all the loss of clonogenicity in irradiated cells. The role of p53 in radiation-induced apoptosis is also discussed, with data presented showing that both p53-dependent and independent pathways for radiation-induced apoptosis exist, depending on the cell type.

Key words: Apoptosis; cell cycle; clonogenicity; p53; radiation.

Introduction

For many years, most studies on cancer focused on the abnormal cell proliferation in tumors, but it is now widely recognized that loss of control of cell death may be as important as increased cell growth in many malignancies. Much of the relevant cell death occurs by apoptosis. It has also become quite clear that not only can apoptosis occur spontaneously in normal and tumor cells, but it can be induced by many anti-cancer agents such as ionizing radiation, UV radiation, many chemotherapeutic drugs, photodynamic therapy and hyperthermia. Ionizing radiation causes apoptosis in many cell types, both normal and cancerous, including, for example, in vivo systems such as small intestine, salivary gland, thymocytes, a murine ovarian carcinoma, and several murine mammary adenocarcinomas, and in vitro systems such as murine lymphoma cells, isolated thymocytes, a murine T-cell hybridoma, murine teratocarcinoma cells and myc and/or ras transfected rat embryo fibroblasts.

Traditionally, it has been thought that ionizing radiation kills cells predominantly by a reproductive mode of cell death, so-called mitotic death, in which the radiation produces DNA damage, residual unrepaird or misrepaired DNA damage results in chromosome aberrations (largely exchanges and interstitial deletions) and genomic instability, and these persistent lesions eventually lead to cell death in the progeny, usually after several mitotic cycles. It was recognized that some cell types, particularly hematopoietic cells such as lymphocytes, undergo a very rapid (within hours) cell death, called interphase death (defined as death of the cell prior to the first post-irradiation cell division), now recognized in lymphocytes to be...
apoptosis. Hence, apoptosis has been viewed as an ‘alternative’, or non-mitotic, cell death. This distinction, however, may be misleading for at least two reasons. First, apoptosis will result in decreased clonogenicity, just as does ‘mitotic cell death’. Thus, the more important question is: does altering the amount of apoptosis change the clonogenic outcome or merely change the mode of cell death? Second, accumulating evidence now makes it clear that apoptosis can occur, not just within a short time after irradiation, but many hours, even days, later, after cells have undergone one, two or even more mitotic cycles, i.e., apoptosis can be the final form of cell demise in ‘mitotic death’. This review will stress these two aspects of radiation-induced apoptosis — the quantitative relationship between apoptosis and loss of clonogenic ability and the time course of apoptosis after radiation exposure. Other interesting and relevant topics related to radiation-induced apoptosis, such as pharmacological intervention and signalling pathways, have been covered in other recent reviews (e.g., 19,20).

Shapes of dose–response curves

Dose–response curves for apoptosis induction in cells irradiated in vitro exhibit one of two general shapes. Some have a sigmoid shape, with initial low doses of radiation causing no or little increase in apoptosis over the background level, followed by a region of markedly increasing apoptosis with dose, then reaching a plateau region where increasing the dose further does not increase the fraction of apoptotic cells. This pattern is illustrated in Figure 1 for human leukaemia HL-60 cells, which are relatively resistant to radiation-induced apoptosis — the quantitative relationship between apoptosis and loss of clonogenic ability and the time course of apoptosis after radiation exposure. Other interesting and relevant topics related to radiation-induced apoptosis, such as pharmacological intervention and signalling pathways, have been covered in other recent reviews (e.g., 19,20).

Time course for appearance of apoptosis after irradiation

Most of the initial studies of apoptosis caused by ionizing radiation focused on cell death that occurred rapidly (i.e., within hours) after radiation