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.

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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).

In vivo, where measurement of apoptosis is usually based on histological assays, the shapes of dose–response curves for radiation-induced apoptosis are similar to those seen in vitro, although the maximum percentage of apoptotic cells observed is usually less, for example in a murine ovarian carcinoma the maximum is approximately 30–35%. This difference may reflect, at least in part, differences in the assays used. The histological assay, used in vivo, measures the level of apoptosis at a single time point after exposure to radiation; hence, cells that have not yet undergone apoptosis or those apoptotic cells which have already been phagocytosed by surrounding cells will not be accounted for, and the per cent apoptotic cells may be an underestimate. On the other hand, many of the assays used in vitro, such as the DNA fragmentation assay, measure cumulative amount of apoptosis up to the time of assay, and, therefore, might be expected to indicate greater levels of apoptosis than would be indicated by a histological assay conducted at a single time point in the same cell line. However, the DNA fragmentation assay suffers from the limitation that the exact relationship between per cent DNA fragmentation and histological apoptosis is not clear: is the percentage of DNA fragmentation proportional to the percentage of cells in the population that have undergone apoptosis, as is usually assumed, or do a larger fraction of cells actually undergo apoptosis, but in those apoptotic cells only a portion of the DNA is fragmented? In a recent, thoughtful commentary, Potten has addressed the questions of the relationships between apoptosis assays and between in vitro and in vivo systems.

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