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Radiation-induced Cell Death and its Mechanisms

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Elucidation of the molecular mechanisms of cell killing induced by ionizing radiation is a main topic in the field of radiation biology. Radiation-induced cell death has been classified as interphase death estimated by vital cell counting, and reproductive death estimated by colony forming assay. Recently, many modes of cell death, such as apoptosis, autophagy, mitotic catastrophe, senescence-like cell death, etc. have been reported. In this review, 1) classification of modes of cell death induced by ionizing radiation, 2) radiation-induced apoptosis and its molecular mechanism, 3) significance of radiation-induced cell death are summarized. Discussion on radiation-induced cell death will also focus on radiation-induced normal tissue damage and strategies in radiation cancer therapy.

Key words: apoptosis, necrosis, autophagy, mitotic catastrophe, senescence-like cell death

It is well-known that ionizing radiation over a certain dose can induce cell killing. However, the detailed mechanism of cell killing is still controversial. Radiation-induced cell killing has been functionally classified into "interphase death" and "reproductive (or mitotic) death". In 1972, the concept of apoptosis, a type of programmed cell death that is defined morphologically, was introduced into the research field. Later then, autophagic cell death also was reported to be involved in radiation-induced cell killing. These cell death modes are gene-regulated. The study of their mediating molecular mechanisms became hot area of research in radiation biology, radiation oncology and radiation emergency medicine with a plethora of reports emerging every year. In this article, the current knowledge on radiation-induced cell killing will be reviewed with special focusing on apoptosis involvement.

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1. Radiation-induced cell death

In the literature of radiation biology, radiation-induced cell death is classified into "interphase death" and "reproductive death". Interphase cell death is the death of irradiated cells before they enter in mitosis and is observed early after irradiation. Reproductive death is the loss of the proliferative ability of the cell. The former has been observed in specific cells, such as peripheral lymphocytes, thymocytes, crypt cells in small intestine, etc., while the latter is considered as the cell death class of most of cells and is observed after several cell division cycles. It is commonly restricted to those cells having an indefinite capacity to divide. Although "reproductive death" indicates loss of potency of cell growth but does not mean "cell death" directly, therefore, cell survival (%) estimated by colony forming units is a cardinal and useful factor to examine

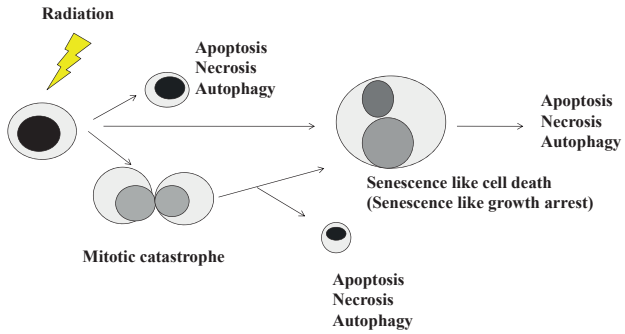

Fig. 1. Modes of radiation-induced cell death.

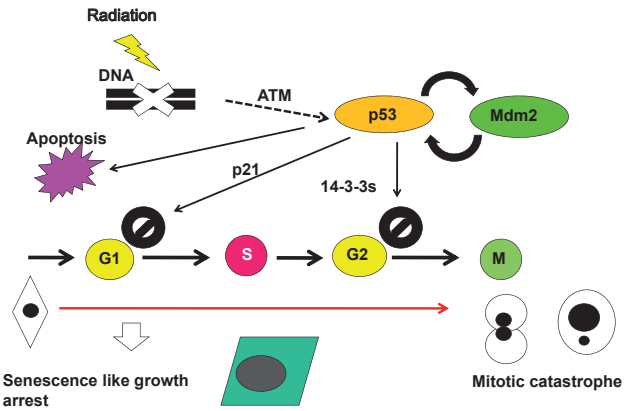

Fig. 2. Relationship between cell cycle checkpoints and cell death mechanism.

radiosensitivity of cancer cells in radiation therapy.

Intracellularly, cell death, however, can be achieved through different molecular pathways each representing a different mode. Necrosis, a type of pathological and irreversible cell death accompanied by the destruction of cell membrane and leakage of intracellular content, has been shown to mediate radiation-induced cell killing. Radiation-induced necrosis was often observed in tissues with inflammation following irradiation at relatively high doses. Pyknosis, a morphological change showing condensation of chromatin in nuclei, has been considered as a typical cell death mode after irradiation for longer periods. In 1972, Kerr et al. has first introduced the concept of apoptosis in Brit. J Cancer[1]. Since then, apoptosis has gained wide interest of many researchers who deliberately studied its underlying mechanisms using different cell lines. Generally, apoptosis induction in cancer cell lines with mutant type of p53 is difficult and takes long time, however, cells such as rodent thymocytes and human leukemia cell lines can still manifest apoptotic features within a relatively short period (several hours) post irradiation, allowing for feasible investigations. Apoptosis is sometimes considered as reproductive death that is linked to mitotic catastrophe and/or senescence like growth arrest.

Mitotic catastrophe is one of the main forms of cell death induced by ionizing radiation. The term 'mitotic catastrophe' is used to denote a mechanism of delayed mitotic-linked cell death. It involves a sequence of events that results from premature or inappropriate entry into mitosis caused by physical or chemical stresses. Mitotic failure is triggered by agents influencing the stability of microtubules and by defective cell cycle checkpoints. Mitotic catastrophe requires normal operation of M phase checkpoint, namely the spindle checkpoint.

Senescence-like growth arrest is the state of irreversible arrest in G1 phase although the cellular metabolism is still going on. Cells in G1 arrest change into multi nucleate cells or giant nucleate cells, and stop their growth afterwards. This abnormal (incomplete) cell division is frequently observed in irradiated cells by long-term microscopic observation. The patterns observed are numerous including binucleate cells, multinucleate cells, polypolar division, etc. Although senescence is usually regarded as cell death, senescent cells can be maintained alive for long time if the culture medium is exchanged continuously, and recently, cases of escaping senescence were reported. Senescent cells can be identified by their positive staining for senescence-associated-β-galactosidase (SA-β-gal).

Autophagy is a process of self-cannibalization. Cells capture their own cytoplasm and organelles and digest them in lysosomes. The resulting breakdown products are fed back to cellular metabolism to be used again in the generation of energy and in building new proteins and membranes. Autophagy preserves the health of cells and tissues by replacing outdated and damaged cellular components with fresh ones. In starvation, it provides an internal source of nutrients for energy generation and, thus, for survival. However, autophagy has been shown recently to contribute significantly to the anti-neoplastic effects of radiation therapy as an alternative form of programmed cell death. Moreover, a "balance" between apoptosis and autophagy induction by radiation has been suggested by some studies[2]. Some pathways, such as the Akt/mTOR or endoplasmic (ER) stress, can possibly switch between both modes. Autophagy is characterized by the activation of autophagosomes formation, LC3 conversion from type I to type II, and the up-regulation of Atg genes.

In Figure 1, we present a scheme for cell death modes induced by ionizing radiation in which apoptosis, necrosis and autophagy are included. When cells are irradiated, the resulting DNA damage is detected by the respective check point and the cell cycle is arrested. If DNA damage can be repaired, the cell cycle will be resumed and cells survive. If DNA damage repair fails, cells will engage in multiple modes of cell death (Fig. 2).

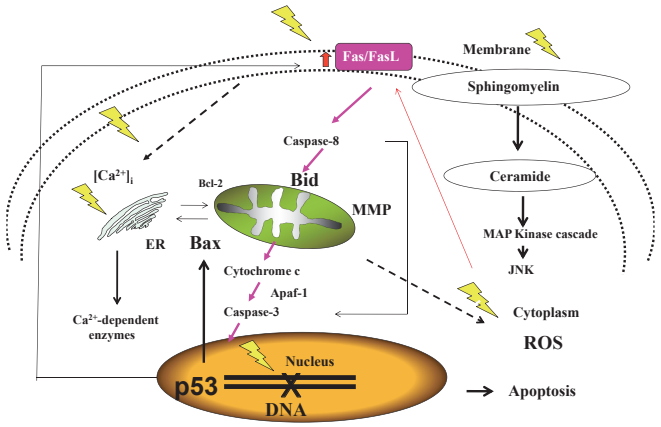

Fig. 3. Molecular mechanisms of radiation-induced apoptosis.

2. Mechanisms of radiation-induced cell death

Apoptotic pathways are classified into an extrinsic pathway, occurs via external death signaling (such as Fas/FasL and their receptors on the cellular membrane), and an intrinsic pathway, controlled by mitochondrial Bcl-2 family proteins. In both pathways, the multistep activation of caspases plays a cardinal role in the execution of apoptosis.

The ionizing radiation, such as X-rays and γ-rays, can interact directly with critical targets in the cells causing ionization or excitation of biological molecules and thus initiating a chain of events that lead finally to biological changes. Alternative to this direct interaction, radiation can interact with other molecules in the cells, e.g. water molecules, to produce free radicals, such as hydroxyl radicals, that can diffuse far enough to reach and damage other critical targets. This is called the indirect action of radiation.

Hydroxyl radicals are highly toxic reactive oxygen species (ROS) which react rapidly with cellular components and cause DNA strand breaks and lipid peroxidation of membranes. These damages subsequently stimulate a cascade of apoptosis signaling.

The use of hydroxyl radical scavengers can suppress radiation-induced cell killing as estimated by clonogenic cell survival assay. When the effect of some antioxidants on X-ray-induced apoptosis in human lymphoma U937 cells were examined 6 hrs post irradiation, no apparent correlation was observed between the concentrations of antioxidants that inhibited DNA fragmentation by 50% and their respective rate constants for scavenging hydroxyl radicals. These results indicate that the prevention of radiation-induced apoptosis by antioxidants cannot be solely attributed to the scavenging ability and that the role of hydroxyl radicals in apoptosis is relatively small[3]. On the other hand, we have studied the possible chemical enhancement of radiation-induced apoptosis. Although the intracellular oxidative stress plays an important role in the modulation of apoptosis, no enhancement was detected upon combining radiation with chemicals producing superoxide anion intracellularly. An enhancement was only observed in combination with chemicals generating peroxides indicating the selective role of the long-lived peroxides, e.g. hydrogen peroxide, in the modification of radiation-induced apoptosis[4-7].

Apoptosis has morphological characteristic features including cell shrinkage, membrane blebbing, chromatin condensation, and nuclear fragmentation. Apoptosis, or programmed cell death, is one of the major control mechanisms by which cells undergo self-destruction if DNA damage is not repaired. As mentioned earlier, apoptosis can be triggered intrinsically or extrinsically by DNA damage or other types of severe cellular stresses such as elevated intracellular ROS. There are many factors involved in modulating radiation-induced apoptosis including 1) p53, a tumor suppressor gene that either arrests the cell cycle or activates apoptosis, 2) ceramide, and 3) calcium ions. In all apoptosis signaling pathways, several intracellular proapoptotic proteins are released and function in activating caspases. These caspases are a network of proteases that ultimately destroy the critical structural proteins in the cell and stimulate DNA fragmentation, culminating in cell death. The increase in intracellular calcium ions concentration ($[Ca^{2+}]i$) activates Ca^{2+}-dependent neutral proteases, calpain, other Ca^{2+}-dependent enzymes, and endonucleases to function in the progression of apoptosis. This increase originates from the Ca^{2+} influx from outside of the cells and its release from the intracellular Ca^{2+} stores in mitochondria and endoplasmic reticulum (ER). The role of Ca^{2+} in radiation-induced apopotosis has been already reported (Fig. 3).

Recently, it has been shown that also autophagy, which is caused by ER stress, contributes to the modulation of radiation-induced cell killing through a balance between apoptosis and autophagy. Our laboratory has demonstrated that apoptosis enhancement could be induced under certain levels of physical and chemical stresses, such as that achieved by the combination between heat and a nitroxide (TEMPO), whereas a conversion from apoptosis to autophagy was observed when all caspases were inactivated under more severe stress through extending the heating time[8]. Both modes of cell death have common signaling pathways and further attention should be paid for considering the balance between them in irradiated cells for better understanding of the mechanisms of radiation-induced cell-death.

3. Implications of radiation-induced cell death

Although the induction of cell killing in cancer cells is a therapeutic advantage, its induction in normal cells

Table 1. Types of cell death

• Apoptosis
• Autophagy and autophagic cell death
• Necrosis
• Mitotic catastrophe
• Anoikis
• Exitotoxicity
• Wallerian degeneration
• Paraptosis
• Pyroptoisis
• Pyronecrosis
• Entosis

during radiation accidents and interventional radiology is responsible for the undesirable side effects, whether acute or late. Radiation-induced injury in human body is a product of the accumulation of serious effects of radiation on each of the cells in exposed tissues and organs, which finally impacts the whole body. These effects can be induced in many cell types by low-dose irradiation without apparent injuries, for example, low dose at several cGy can cause apoptosis in crypt cells in small intestine after several hours. However, no lethal damage was observed at a dose less than 10 Gy since radio-resistant stem cells grow and replace the damaged cells. By eliminating all the cells with damaged DNA, the risk of genetic damage is reduced and as such apoptosis can be considered as a self-defense mechanism working against carcinogenesis and the growth and differentiation of cells with abnormal DNA. This is especially critical for the suppression of radiation-induced teratogenesis upon exposure to radiation during the process of fertilization to embryogenesis to keep a normal progeny. The elimination of cells possessing DNA damages was shown to occur through p53-dependent apoptosis[9]. However, when apoptosis induction exceeds a certain limit, it can cause tissue injury and dysfunction.

There are many other phenomena observed in response to irradiation, including morphological changes of mitochondria, a decrease in the number of mitochondria, a decrease in the shape of spindle, and an increase in the number of centrosomes, etc. It is still unclear whether these observations are causes or consequences of cell death.

Finally, it is noteworthy to mention that the whole picture of cell death pathways is still beyond our sight and that current research should ensue to investigate these pathways especially for the newly discovered types of cell death based on morphological distinction. Recently, the Nomenclature Committee on Cell Death (NCCD) proposed unified 11 criteria (see Table 1) for the definition of cell death and its different morphologies[10]. Moreover, the involvement of such types in radiation-induced cell killing should attract the interest of radiation expertise in the future.

References

1. Kerr JFR, Wyllie AH, and Currie AR (1972) Apoptosis: a basic biological phenomenon with wide ranging implication in tissue kinetics. Br J Cancer 26: 239-257.
2. Moretti L, et al. (2007) Switch between apoptosis and autophagy. Radiation-induced endoplasmic reticulum stress? Cell Cycle 6: 793-798.
3. Cui Z-G, et al. (2004) Effects of antioxidants on X-ray-or hyperthermia-induced apoptosis in human lymphoma U937 cells. Apoptosis 9: 757-763.
4. Cui Z-G, et al. (2004) Enhancement of radiation-induced apoptosis by 6-formylpterin. Free Radical Res 38: 363-373.
5. Yu D-Y, et al. (2008) Enhancement of hyperthermia-induced apoptosis by a new synthesized class of benzocycloalkene compounds. Apoptosis 13: 448-461.
6. Yu D-Y, et al. (2007) Enhancement of hyperthermia-induced apoptosis by a new synthesized class of furan-fused tetracyclic compounds. Apoptosis 12: 1523-1532.
7. Yu D-Y, et al. (2009) Enhancement of radiation-induced apoptosis of human lymphoma U937 cells by sanazole. Apoptosis 14: 655-664.
8. Zhao Q-L, Fujiwara Y, and Kondo T (2010) Synergistic induction of apoptosis and caspase-independent autophagic cell death by a combination of nitroxide Tempo and heat shock in human lymphoma U937 cells. Apoptosis 15: 1270-1283.
9. Norimura T, et al. (1996) p53-dependent apoptosis suppresses radiation-induced teratogenesis. Nat Med 2: 577-580.
10. Kroemer G, et al. (2009) Classification of cell death: recommendations of the Nomenclature Committee on Cell Death. Cell Death Differ 16: 3-119.