Apoptosis in immune cells induced by fission fragment ^{147}Pm*

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Abstract Apoptosis in human acute lymphoblastic leukemia cell line Molt-4 cell and macrophage cell line Ana-1 cell could be induced by fission fragment ^{147}Pm. The cumulative absorption dose of ^{147}Pm in cultural cells through different periods were estimated. By using fluorescence microscopy and microautoradiographic tracing it can be found that Molt-4 and Ana-1 cells internally irradiated by ^{147}Pm, displayed an obvious nuclear fragmentation and a marked pyknosis in immune cell nuclei, as well as DNA chain fragmentation and apoptotic bodies formation. The microautoradiographic study showed that ^{147}Pm could infiltrate through cell membrane and displayed membrane-seeking condensation in cells. At the same time, the membrane-bounded apoptotic bodies were observed. Experimental results in recent study provide evidence that Molt-4 and Ana-1 immune cells undergo apoptosis while internally irradiated with ^{147}Pm.

Keywords Fluorescence microscopy, Microautoradiography, Apoptosis, Molt-4 cell, Ana-1 cell, Fission fragment ^{147}Pm, Cell culture

1 Introduction

Up to date, fission fragment ^{147}Pm has been considered for use as radioactive heat sources for light-weight electrical power units[1] and as energy source in luminescent paints for watch and instrument dials.[2] Large scale separation of ^{147}Pm from fission product waste and preparation of material for industrial application constitute a potential source of radiation exposure to workers in this field.[3] Therefore, people are paying close attention to studies of the immunotoxicity in the body induced by fission fragment ^{147}Pm. And it is necessary to study apoptosis[4] in immune cells induced by internal irradiation with ^{147}Pm. So in this paper, apoptosis in Molt-4 and Ana-1 cells were studied by fluorescence microscopic and microautoradiographic observations with internal irradiation of ^{147}Pm.

2 Experimental method and results

2.1 Cell culture

Molt-4 cell, a human acute lymphoblastic leukemia cell line and Ana-1 cell, a macrophage cell line stemming from Shanghai Immunological Institute, were maintained in RPMI 1640, supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin, 2 mmol/L L-glutamine, as well as 5×10^{-5} mol 2-mercaptoethanol, which was known as the complete RPMI 1640 medium. Cells were kept in an atmosphere containing 0.05 volume fraction CO_2 at 37°C and used when in exponential growth. Then, Molt-4 and Ana-1 cells, internally irradiated with fission product ^{147}Pm, were washed three times with Ca^{2+} and Mg^{2+} free Hanks solution, then adjusted to a concentration of 2×10^6 cells/ml in complete RPMI 1640 medium.

2.2 Absorption dose estimation of fission fragment ^{147}Pm in cultural cells

$^{147}Pm(NO_3)_3$ with radioactive and chemical purity was used in this work. First, 1 ml of fission fragment ^{147}Pm solution with radioactivities 7.4×10^2 kBq/ml in complete RPMI 1640 medium was added into Molt-4 and Ana-1 cell suspensions at 2×10^6 cells/ml; then incubated in a 5% CO_2 atmosphere at 37°C through 3, 6, 9, 12, 24 and 48 h. Therefore the cumulative absorption dose can be calculated according to the following formula[5]

$$D = AE/m$$

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here D is cumulative absorption dose in cultural cells, mGy; A radioactivities, Bq; E average energy of β particles, MeV; m mass of the cells. So that, the cumulative absorption dose through different periods of internal irradiation with ^{147}Pm may be calculated: 40.8 mGy/3 h, 81.8 mGy/6 h, 122.8 mGy/9 h, 163.7 mGy/12 h, 327.4 mGy/24 h and 654.7 mGy/48 h.

2.3 Fluorescence microscopic observation

Molt-4 and Ana-1 immune cells were suspended in complete RPMI 1640 medium to form suspension at 2×10^6 cells/ml. The cell suspensions of 1 ml were added to 24-well microtitration plate. Then 1 ml of fission fragment ^{147}Pm with 3.7×10^2 kBq/ml also in complete RPMI 1640 medium was added to each experimental well. To control wells 1 ml RPMI 1640 medium was added only. The microtitration plates were then incubated in a 5% CO_2 at 37°C through 3, 6, 9, 12, 24 and 48 h. After internal irradiation with ^{147}Pm, harvest Molt-4 and Ana-1 cells, and wash 5 times with Hanks solution to take away the free radioactivities of ^{147}Pm. After a while, Molt-4 and Ana-1 cells were suspended into hypotonic fluorescent solution consisting of 50 mg/ml pyridine iodide and 0.001 mass fraction sodium acetate as well as 0.001 mass fraction triton X-100[6], in order to examine the morphology of Molt-4 and Ana-1 cells' nuclei.

Experimental results indicated that internal irradiation of ^{147}Pm could induce nuclear fragmentation, nuclear pyknosis, DNA chain fragmentation, and apoptotic bodies formation in Molt-4 and Ana-1 cells as shown in Fig.1.

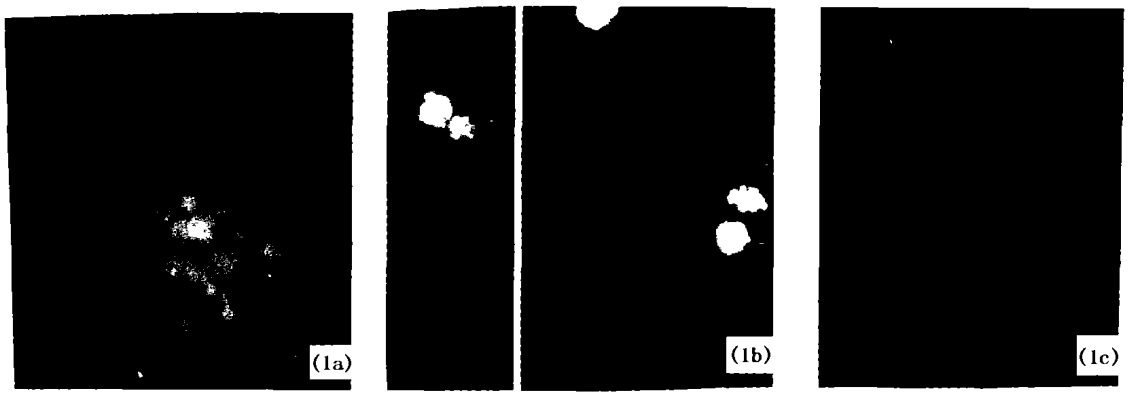

Fig.1 Fluorescence microscopic appearance of PI-stained Molt-4 cell nuclei after 12 h incubation with 3.7×10^2 kBq/ml ^{147}Pm (1a); PI-stained Ana-1 cell nuclei after 24 h incubation with ^{147}Pm (1b); and PI-stained Ana-1 cell nuclei after 12 h incubation with ^{147}Pm (1c); ×1250.

Fig.1a gives a marked reduction of cell nuclei in diameter and fragmentation; Apoptotic cells in Fig.1b are identified by their pyknosis and fragmented nuclei; Fig.1c gives the fragmentation of nuclei and apoptotic bodies formation

2.4 Microautoradiographic observation

In microautoradiographic study Molt-4 and Ana-1 cells were incubated with fission fragment ^{147}Pm in a 5% CO_2 atmosphere at 37°C through 3, 6, 9, 12, 24 and 48 h. In detail, put 20 μl Molt-4 or Ana-1 irradiated cells on microscopic slides. Then coated with thin collodion membrane. Experimental slides were smeared with type N-4 liquid nuclear emulsion, which was 1:1 diluted with double distilled ion free water, put into 10% stable reagent 6-nitrobenzene miazol.[7] The slides were then allowed to be exposed in dry nitrogen ambient for 15 d at 0°C. After 15 d of exposure, the emulsion-coated sections were developed and fixed at 18°C for 12 min. Thereafter, washed with running water and dipped in 5% glycerol solution. The sections were soon double stained with hema-

toxylin and eosin in reformable method.[8] Relative regional autoradiographic activity was then determined by visualizing autoradiographic activity tracks in internally irradiated immune cells.

The microautoradiographic tracing shows that ^{147}Pm could infiltrate through cell membrane as shown in Fig.2a, induce membrane-seeking condensation as shown in Fig.2b; and induce the memberane-bounded apoptotic bodies as shown in Fig.2c.

Fig.2 Autoradiography of Molt-4 cell incubated for 6 h with ^{147}Pm (2a), and Ana-1 cell incubated for 3h(2b), 6 h(2c) with ^{147}Pm

3 Conclusion

Fluorescence microscopy study shows that the Molt-4 and Ana-1 immune cells, internally irradiated by ^{147}Pm, displayed an obvious nuclear fragmentation and a marked pyknosis in immune cells nuclei, as well as DNA chain fragmentation and apoptotic bodies formation in apoptotic cells.

The microautoradiographic tracing demonstrates that ^{147}Pm could infiltrate through cell membrane and induce membrane-seeking condensation in immune cells. At the same time, the membrane-bounded apoptotic bodies are observed.

Experimental results indicate that apoptotic cells formation in Molt-4 and Ana-1 immune cells induced by fission fragment ^{147}Pm were dependent on the ^{147}Pm-treated time as well as accumulated absorption dose.

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