DNA gel electrophoretic and microaut oradiographic studies on apoptosis in bone tumor cells after exposure with ^{153}Sm-EDTMP*

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Abstract The apoptosis in bone tumor cells is studied after ^{153}Sm-EDTMP irradiation. Fragmented DNA is analyzed by agarose gel electrophoresis. Experimental observations show that ^{153}Sm-EDTMP exposure induces the internucleosomal DNA damage in bone tumor cells the DNA ladder pattern formation in bone tumor cells is shown. At the same time, the microautoradiographic study indicates that ^{153}Sm-EDTMP could permeate through cell membrane and displays membrane-seeking condensation in bone tumor cells. Soon afterwards ^{153}Sm-EDTMP could be phagocytized by the tumor cells and distributed in cytoplasm as well as nucleus in the form of phagosome. With the prolongation of observing time, the membrane-bounded apoptotic bodies are observed.

Keywords DNA gel electrophoresis, Microautoradiography, Apoptosis, Bone tumor cells, Irradiation, ^{153}Sm-EDTMP

1 Introduction

At present, the favourable characteristics of radionuclide ^{153}Sm-EDTMP permit optimal internal radiotherapy for clinical application.[1] Now ^{153}Sm-EDTMP (ethylenediaminetetramethylene phosphonate) has been used in patients requiring analgesia for pain arising from disseminated skeletal metastasis unresponsive to all appropriate conventional treatment modalities.[2] ^{153}Sm-EDTMP is effective not only in alleviating the pain of disseminated skeletal metastasis, but also in treatment of recurrent pain.[3] But up to date, its mechanism in the treatment of painful skeletal metastasis is still unknown.[4] Therefore, we paid attention to study apoptosis of bone tumor cells induced by ^{153}Sm-EDTMP with DNA gel electro- phoretic and microautoradiographic observations.

2 Experimental methods

2.1 Cell culture conditions

A human bone tumor cell line HOS-8603 stemming from Cellular Imunological Center of Suzhou Medical college was harvested and maintained in RPMI 1640 (GIBCO) supplemented with 10% fetal calf serum, 100mg/L penicillin, 100mg/L streptomycin, 2 mmol/L L-glutamine as well as 5×10^{-5} mol of 2-mercaptoethanol, which was known as the complete RPMI 1640 medium. The tumor cells were kept in an atmosphere containing 5% CO_2 at 37°C and used when in exponential growth.[5] Cells were harvested from exponential-phase maintenance cultures using trypsin:versene (0.05:0.02%) treatment of monolayer cultures. Thereafter, the bone tumor cells were washed three times with Ca^{2+} and Mg^{2+} free Hanks solution. Single cell suspensions were prepared with complete RPMI 1640 medium and the cells were counted using a hemocytometer. Finally, the suspensions were adjusted to a concentration of 2×10^6 cells/mL.

^{153}Sm-EDTMP with radioactive and chemical purity was used in this study. The bone tumor cells suspensions at 2×10^6 cells/mL was added into 1 mL of ^{153}Sm-EDTMP solution with a radioactivity of 3.7×10^2 kBq/mL in complete RPMI 1640 medium in a 24-well microtitration plate. Then the cells were incubated in a 5% CO_2 atmosphere at 37°C for 3, 6, 9, 12 and 24 h with ^{153}Sm-EDTMP.

2.2 Cellular DNA extraction and agarose gel electrophoretic observations

Cellular DNA of 2×10^6 bone tumor cells after 3,6, 9 and 12 h period irradiation with

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^{153}Sm-EDTMP was obtained by extraction with 1 mL buffer solution containing 10 mmol tris·HCl (pH 8.0), 1 mmol EPTA, 10 mmol NaCl,1% SDS, 20μg RNase and 100μg proteinase K (Merck, Germany). Cells were kept in 37°C through 12 h and cooled down to room temperature. Then saturated phenol solution was added with the same volume. Mixed solution was isolated centrifugally with 12,000 g in 10 min. Thereafter, drew apart the supernatant liquor and put into the Eppendorf centrifuge tube. Speciments were extracted again with phenol:chloroform 1:1 solution, then added 0.3 mol/L sodium acetate with dehydrated ethanol at 4°C for centrifugal extraction in 10 min. Extracted DNA was washed twice in 70% ethanol, and dried at routine temperature. Finally, DNA was dissolved in 1 mmol EDTA with 10 mmol tris·HCl (pH 7.8) TE buffer solution, and was kept at 4°C.

Agarose gel electrophoresis made by 1.5% agarose gel containing 0.5 mg/L ethidium bromide and 20μg/L extracted sample containing 20μg DNA mixed with 0.25% bromophenol blue and 40% sucrose were then put into gel electrophoretic slot at 50 V for 1.5 h electrophoretic analysis. The gel sample was photographed under UV light.

2.3 Microautoradiographic observations

Now microautoradiographic study is carried out on bone tumor cells internally irradiated with ^{153}Sm-EDTMP for 3, 6, 9, 12 and 24 h in a 5% CO_2 atmosphere at 37°C. The procedures are as follows: 20μl each of control and experimental bone tumor cells were mounted separately on microscopic slides, then coated with thin collodion membrane. Thereafter, the slides were smeared with type N-4 liquid nuclear emulsion, which was 1:1 diluted with double distilled water, and put into 10% stable reagent 6-nitrobenzene miazol.[6] The slide samples were then allowed to be exposed to dry nitrogen condition for 3 d at 0°C. After 3 d in dark exposure, the emulsion-coated sections were developed and fixed at 18°C for 12 min. Soon afterwards, the slides were washed with running water and dipped in 5% glycerol solution. The sections were soon double stained with hematoxylin and eosin with reformed method.[7] Relative regional autoradiographic activity was then determined by visualization of the autoradiographic activity tracks in irradiated bone tumor cells.

3 Results

3.1 DNA gel electrophoretic observations

Agarose gel electrophoresis illustrated the DNA fragmentation such as ladder pattern formation in bone tumor cells, irradiated by ^{153}Sm-EDTMP after different intervals as shown in Fig.1. The internucleosomal DNA damage in bone tumor cells were readily detected 9 h after exposure with ^{153}Sm-EDTMP, in which a typical ladder of DNA was seen. In comparison, no obvious DNA ladder in bone tumor cells treated with ^{153}Sm-EDTMP less than 6 h was observed.

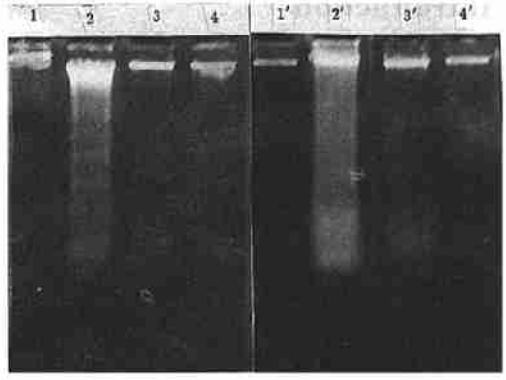

Fig.1 DNA ladder pattern formation in bone tumor cells given by agarose gel electrophoresis
Lanes 1, 1′, control. Lanes 2, 3, 4, 2′, 3′, 4′ Cells irradiated by ^{153}Sm-EDTMP after 12, 9, 6, 24, 9, 3 h

3.2 Microautoradiographic study

The microautoradiographic visualization indicated that ^{153}Sm-EDTMP could penetrate through bone tumor cells membrane as shown in Fig.2a, could be phagocytized by bone tumor cells as shown in Fig.2b, can be distributed in cytoplasm and nucleus in the form of phagosome as shown in Fig.2c also can be observed in membrane-bounded apoptotic bodies as shown in Fig.2d.

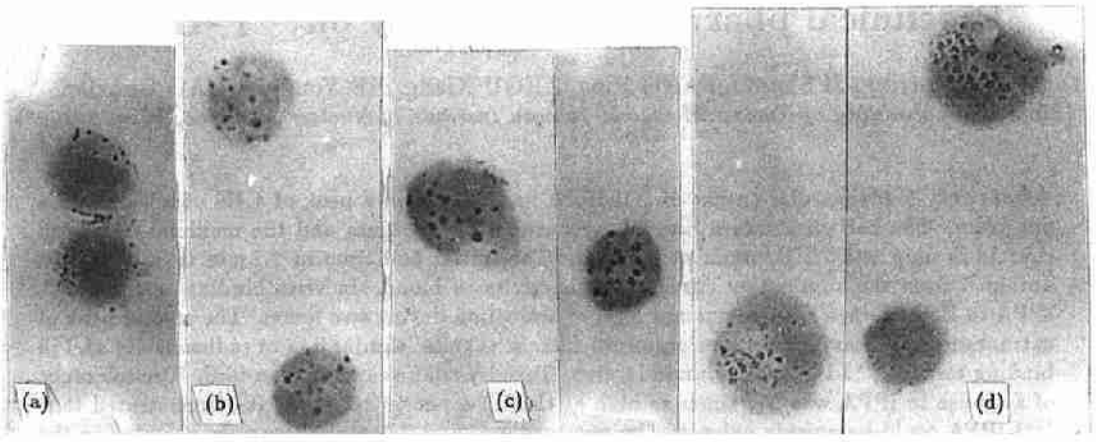

Fig.2 Microautoradiography of bone tumor cells irradiation with ^{153}Sm-EDTMP after 6 h(a), 9 h(b), 24 h(c) and 24 h(d)*

*The tracks are obserred in membrana-bounded apoptotic bodies, × 2000

4 Discussion

Previous study directed towards external radiation induced apoptosis in cells.[8] But, in present study, bone tumor cells were irradiated with ^{153}Sm-EDTMP. The internucleosomal fragmentation of DNA in bone tumor cells, which resulted in a ladder type pattern comprising 180 base pair intervals in agarose gel electrophoresis, was a key molecular event in apoptosis. Recent data provided strong evidence that bone tumor cells undergo apoptosis when encountered ^{153}Sm-EDTMP. In the meantime, the microautoradiographic study showed that ^{153}Sm-EDTMP could permeate through cell membrane and displayed membrane-seeking condensation in bone tumor cells. Thereafter, ^{153}Sm-EDTMP could be phagocytized and distributed in cytoplasm and nucleus in the form of phagosome. At the same time, the membrane-bounded apoptotic bodies were observed. Our experimental results indicated that progression of apoptosis in bone tumor cells induced by ^{153}Sm-EDTMPwas dependent on the ^{153}Sm-EDTMP exposure time.

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