Experimental Study on Apoptosis of Huan Prostate Cancer Cell Induced by Radionuclide ^{188}Re

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Abstract **Objective** To study the apoptosis of human prostate cancer PC-3 cell induced by ^{188}Re and expression of bcl-2 gene and bax gene. **Methods** Light microscope, transmissional electron microscope, flow cytometer and immunohistochemical method were used to observe the apoptosis of PC-3 cell after being exposed to ^{188}Re of different doses and the expression of bcl-2 gene and baxgene. **Results** ^{188}Re can induced PC-3 cell producing typical morphologic and physiologic-chemical changes of apoptosis and with the rise of radiation dose, apoptosis rate increased, expression of bcl-2 gene decreased and that of bax gene was enhanced. Cells were blocked in G2/M period. **Conclusion** Radionuclide ^{188}Re can induce PC-3 cell apoptosis, which takes on dose-effect relation and cycle-dependance. bcl-2 gene and bax gene play important role in the course.

Key Words ^{188}Re; PC-3 cell; apoptosis; bcl-2 gene; bax gene

Wide-spreading bone metastasis often occur in prostate cancer and its treatment is a difficult question facing clinical doctors. Recently, as the development of nuclear medicine, radionuclide is regarded as an effective therapeutic method for bone metastasis, becoming hot-spot of research[1, 2]. ^{188}Re is a novel therapeutic radionuclide developed in recent years. ^{188}Re not only emits 2.12Mev β-ray suiting therapy, but also emits 15% 155Kev γ-ray fitting imagination. Its half-life is 16.9 hours. ^{188}Re is produced by ^{188}W-^{188}Re generator, prepared and marked simply, which makes baseunit hospital launch radionuclide therapy conveniently. Now, ^{188}Re has been widely used in bone mestastasis and receives good results[3, 4]. However, few research on basic therapeutic mechanism of ^{188}Re has been reported. In this study, we observed the apoptosis of human prostate cancer PC-3 cell induced by radionuclide ^{188}Re in vitro and also expored its mechanism. We hope this study may offer theoretic basis for clinical therapy.

MATERIAL AND METHODS

Cell cultivation and radiation condition

Human prostate cancer PC-3 cell line was from molecular-biology center of the first hospital of xi'an jiaotong university. PC-3 cells were cultured in RPMI medium 1640 involving 10% bovine serum. ^{188}Re was produced by ^{188}W-^{188}Re generator (production of Shanghai Kexing Co.) in form of $^{188}ReO_4^-$ and was concentrated in 2ml physiological saline. Approaching experiment, cells in exponent growth stage were digested with trypsase and prepared cell suspension of certain concentration. 188Re was diluted to diffrent concentration(30, 60, 120, 240 $MBq \cdot mL^{-1}$). The experiment concluded experimental group and control group. ^{188}Re of diffrent doses were added into experimental groups and the same volume RPMI medium 1640 went into control groups. Having reacting for 24 hours, we collected cells and researched.

Morphological observation

Common light microscope observation: Experiment groups and control groups were collected, fixed with 95% alcohol, HE staining, then observing morphological changes under common light microscope.

Transmissional electron microscope observation

Collecting corresponding groups' cells, washed in PBS, fixed with 2% glutaraldehyde and 1% osmium acid, making ultrathin sections, then observing cells' ultrastructure under transmissional electron microscope.

Flow cytometer analysis

Collecting cells, washed in PBS, fixed with pure

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ethanol for 20 min, joining PI dyed liquid, avioding light and reacting for 15 min, then detecting DNA content and cell cycle distribution by means of flow cytometer strument.(FACScan type, production of Coulter Co. in Amercian)

Immunohistochemical dyeing of bcl–2 genes and bax genes

Collecting cells of experimental groups and control groups, operating according to direction of SABC reagent box(production of Fuzhou Maixin Co.), setting up negative control group, selecting five high time views by random under microscope and detected light density value using TD-2000 type pathology image analysis instrument(production of Beijing Tiandi Technology Co.), then got average value ($\bar{x}\pm S$).

Statistics analysis

We adapt SPSS(10.0)software and utilized T test among groups.

RESULTS

Morphologic observation

Observation under light microscope: PC-3 cells showed typical apoptosis morphological character after being exposed in 60MBq·mL^{-1} ^{188}Re for 24 hours. Cells became round and small, Cytoplasm was dyed red, Nucleus pyknosed and smashed, Chromosome was dyed navy blue or blue-black, Apoptosis bodies formed through exocytosis and gemmation. Normal PC-3 cell was in shape of shuttle, its nucleus was regular and was dyed in uniformed blue.

Observation under transmissional electron microscope

Compared with normal cells, apoptosis cells became small, Cytoplasm condensed, Intracellular vocuole increased; Chromatin concentrated or chipped into anomaly masses, Cell organs were conservated well; Nucleus membrane was integnity, Apoptosis bodies could also be seen, which packed integrated cell organs and cell fragments (Fig. 1 and 2).

Flow cytometer detecting apoptosis apex and distributing of cell cycle

Subdoubled apex appeared ahead of G_0/G_1 apex in experiment groups, which was called apoptosis apex (Fig.4), but in control group, there had no subdoubled apex(Fig.3). As dose of ^{188}Re added, apoptosis rate increased accordingly. Cells were blocked in G2/M period. (Tab. 1)

Expression of bcl–2 gene and bax gene

The results of immunohistochemical dyeing in our experiment showed that bcl-2 gene and bax gene were distributed mainly in cytoplast, taking on tiny grain state. With dose of ^{188}Re increasing, expression of bax gene enhanced and that of bcl-2 gene weakened. (Tab. 2)

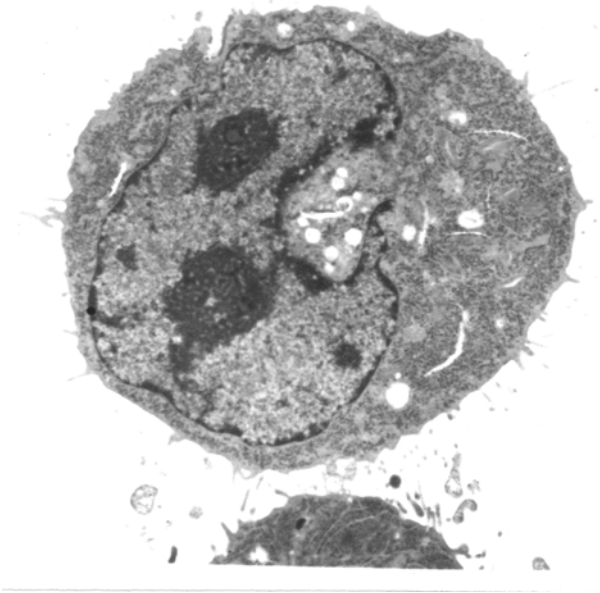

Fig. 1 Normal PC-3 cell (×5000)

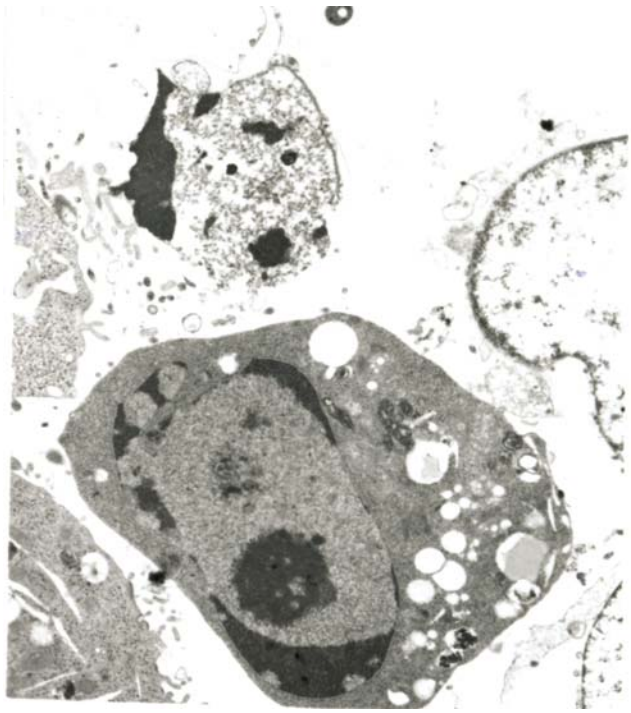

Fig. 2 Apoptosis PC-3 cell, Chromatin concentrated and gathered at intramargin of nuclear membrane (×5000)

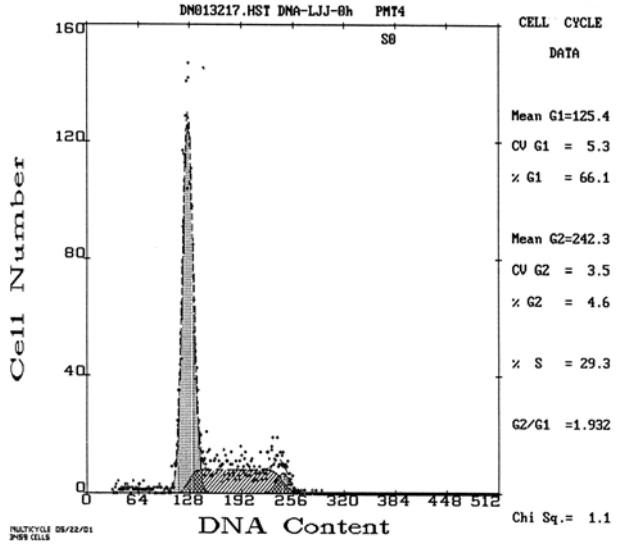

Fig. 3 Without 188Re effection, there has no apoptosis apex in control groups.

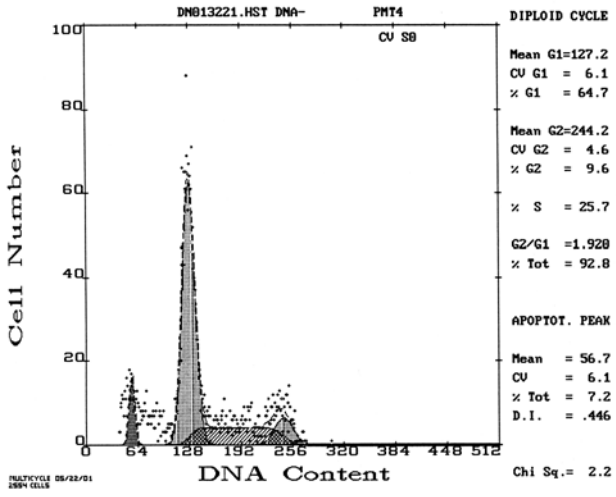

Fig. 4 After exposure to ^{188}Re of 120 MBq·mL^{-1} for 24h, apoptosis apex appeared before G_0/G_1 period.

Table 1. Effection on apoptosis and cell cycle after ^{188}Re of diffrent doses reacting on PC-3 cell for 24h

Concentration of ^{188}Re (MBq·mL^{-1})	Apoptosis rate (%)	Distribution of cell cyle(%)		
		G_0/G_1	S	G_2/M
0	2.3	64.4	27.3	8.3
30	3.7	63.8	26.7	9.5
60	7.2	61.3	24.9	13.8
120	15.8	62.4	22.2	15.4
240	19.4	64.5	20.3	15.2

Table 2. Effection on expression of bcl-2 gene and bax gene after ^{188}Re of diffrent doses acting on PC-3 cells for 24h.

Concentration of ^{188}Re (MBq·mL^{-1})	Average light density values	
	bcl-2	bax
0	0.18±0.04	0.13±0.03
30	0.17±0.06	0.16±0.03
60	0.13±0.05	0.24±0.06
120	0.07±0.02	0.29±0.05
	0.06±0.02	0.31±0.06

Note: P<0.05 compared with control group.

DISSCULTION

Apoptosis is one form of cell death, which is a response of cells aiming at special changes of circumstance factor. Proper dose of ionizing radiation can induce cell apoptosis[5]. Compared with extra radiation, inner radition of radionuclide showed durative of action time and acumulation of effect. Radionuclide can induce cell apoptosis or produce cell necrosis. Different radionuclide, diffrent dose and acting on different kind of

cells may bring different effects. The results of this study demonstrated that radionuclide ^{188}Re might induce human prostate cancer PC-3 cell producing typical morphological changes of apoptosis and with dose adding, apoptosis rate increased accordingly, which may offer help for clinical treatment with proper dose and radition manner at proper time.

The sensitivity of multiplicated cell to radial depends on cell cycle, and diffrent type of cell may have diffrent sensitivity cycle to the same radionuclide[6]. This study showed PC-3 cells were blocked in G_2/M period after being exposed to ^{188}Re. Therefore, combinating cell block and apoptosis, curtailing G_2/M period block with drug, we can increase cell apoptosis and improve radionuclide's therapy effect[7].

Process of cell apoptosis is regulated by many genes. Bcl-2 gene and bax gene play important role in cell apoptosis. Enhancing the expression of bax gene can accelerate cell apoptosis and the increased expression of bcl-2 gene may inhibite cell apoptosis[8]. Our study indicated that as ^{188}Re dose increased, expression of bcl-2 gene fell and that of bax gene enhanced. This is meaningful for tumor treatment by adjusting genes expression [9,10].

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