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Activation of apoptotic pathway via DNA damage by ^{188}Re in non-small cell lung carcinoma, Glioblastoma and Hepatocellular Carcinoma Cells

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1. Introduction

^{188}Re has a variety of interesting therapeutic applications. ^{188}Re has several advantages compared to other beta-emitting radionuclides. It emits beta particles with an average energy of 764 keV and a 155 keV gamma ray, which are suitable for therapy and imaging. Chemical properties of technetium and rhenium are quite similar, and chemical techniques which have been developed for ^{99m}Tc ligands can be applied for ^{188}Re ligands. Easy availability and low cost are the most important merits of ^{188}Re due to the development of ^{188}W/^{188}Re generator. Also, ^{188}Re can be easily obtained in a radionuclidically pure form. The excellent radionuclide purity, availability in relatively high specific activity and the advantages make ^{188}Re a potential and attractive radionuclide for cancer therapy.

In this study we examined was cytotoxic and apoptotic effect of ^{188} Re-mediated beta irradiation on Calu6, T98G and Hep3B cancer cells. These findings demonstrate that beta irradiation induced apoptosis by DNA damage.

2. Methods and Results

2.1 Cell lines and culture condition

The glioblastoma cell line T98G, the non-small cell lung carcinoma cell lines Calu6 and hepatocellular carcinoma cell line Hep3B were grown as monolayers and maintained at 37°C in a humidified incubator with 5% CO_2 in Modified Eagle's Medium supplemented with 10% FBS and 1% antibiotics (GIBCO BRL, Paisley, UK).

2.2 Radioisotope production

^{188}Re was eluted from the ^{188}W generator(Polatom). ^{188}W($T_{1/2}$ =69.4 d) decays to ^{188}Re($T_{1/2}$ = 17.02 h, E $E_{\beta(max)}$ = 2.12 MeV, E_γ = 155 keV).

2.3 Cytotoxicity Assay.

The cell viability was determinated by the MTT assay. Calu6, T98G and Hep3B(5×10^4cells/well)cells were incubated with various activities of ^{188}Re (0 to 1,500 uCi) for 48h. 20 ul MTT(5 mg/ml) stock solution were added to each well for 2 h incubation at 37°C, and then 100 µl of the solubilization buffer were added. The absorbance was measured at 570 (ref. 690) nm using a microplate reader. The cell viability was calculated as the ratio of absorbance in wells containing ^{188}Re compared with control (con) cells. IC_{50} values were calculated from a linear regression from dose-dependent curves of at least five points. Values are means (±S.D.) of 3 experiments.

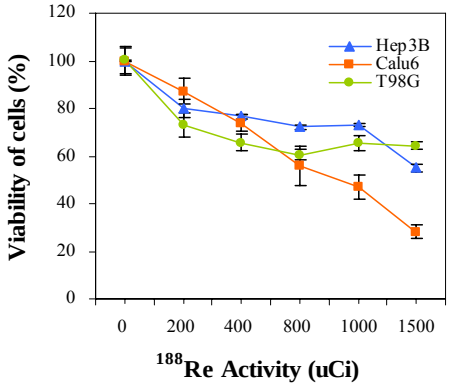

Fig. 1. *The effect of ^{188}Re activity on lung, brain and liver cancer cells. Cells were incubated with various activities of ^{188}Re (0 to 1,500 uCi) for 48 h. Cell viability was determined by the MTT assay. Values are means (±S.D.) of 3 experiments. *Compared with control P < 0.01 (Student's t test).*

2.4 Cell Proliferation Assay.

Cell proliferation was determined by measuring the levels with a BrdU assay kit purchased from Chemicon. according to the manufacturer's instructions. Briefly, 5×10^4 cells/well were seeded in standard 96-well microtiter plates and treated with various activity of ^{188}Re. The cell proliferation was calculated against the ratio of absorbance of each well.

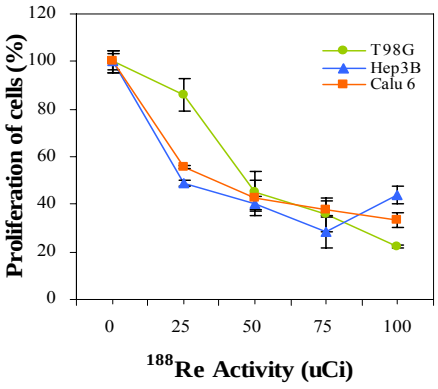

Fig. 2. *The effect of ^{188}Re activity on lung, brain and liver cancer cells. Cells were incubated with various activities of ^{188}Re (0 to 100 uCi) for 48 h. Cell proliferation was determined by the Brdu assay. Values are means (±S.D.) of 3 experiments.*

Table 1. *The IC_{50} values of ^{188}Re on cancer cells after 48 h of treatment. Calculation was made from a linear regression of dose-dependent curves of at least five points.*

	^{188}Re			
	IC_{50} (uCi)	Dose Lethal (Gy)	IC_{50} (uCi)	Dose Lethal (Gy)
Calu 6	49	116	973	2306
Hep3B	42	101	1693	4011
T98G	59	141	1870	4431
	Based on BrdU assay		Based on MTT assay	

2.5 Determination of Cell Death

Cleavage of genomic DNA during apoptosis may yield double stranded, low molecular weight DNA fragmentas as well as single strand breaks("nicks") in high molecular weight DNA. Those DNA strand breaks can be identified by labeling free 3'-OH terminal with modified nucleotides in an enzymatic reaction. We confirm that the apoptotic effect of ^{188}Re activity on calu 6, Hep3B and T98G. Cells were incubated with ^{188}Re activity of IC_{50} based on MTT and BrdU assay for 48h. Apoptotic cell death was determined by the TUNEL method using an assay kit (Roche Applied Science). And then fixed cells were observed under a standard fluorescence microscope.

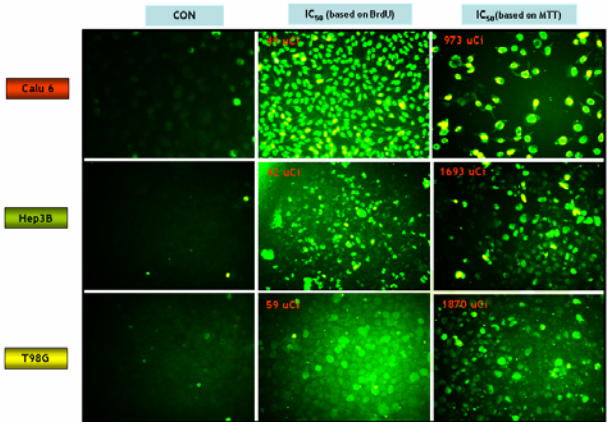

Fig. 3. *The apoptotic effect of ^{188}Re activity on lung, brain and liver cancer cells. Cells were incubated with activities of ^{188}Re based on MTT and BrdU assay for48h. Apoptotic cells were analyzed by TUNEL assay.*

3. Conclusion

Our data suggest that the non-small cell lung carcinoma, glioblastoma and hepatocellular carcinoma cells exposed to ^{188}Re display diminished cellular viability. Thus, ^{188}Re is potential ^{188}Re labeled radiopharmaceuticals that are more specific for target lesions such as cancer specific monoclonal antibodies and peptides. It is believed that ^{188}Re generator system will bring about a renaissance in radionuclide therapy, in a way similar to that of ^{99m}Tc generator and nuclear imaging. However, we have to demonstrate that ^{188}Re activated apoptosis signaling pathways.

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