Preparation for RNA-sequencing of ^{211}At-MABG treated neuroblastoma cell lines

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Pheochromocytomas (PCCs) are rare neuroendocrine tumors with malignant progression, and reported strong anti-tumor effects of α-emitting *meta*-^{211}At-astato-benzylguanidine (^{211}At-MABG) in a PCC mouse model, suggesting a potential option for targeted α therapy (TAT) for patients with malignant PCC,[1] that the gene expression profiles of cell cycle checkpoints displayed similar modes of cell death via the p53-p21 signaling pathway after ^{211}At-MABG treatment and γ-rays irradiation.[2] On ^{211}At-MABG therapy, p53-p21 signaling is an important cell-killing pathway induce reproductive cell death, *e.g.* cell cycle arrest. However, in p53 mutated neuroblastoma cell line, ^{211}At-MABG also showed cell-killing effects.[3] It is possible that there are other important pathways in ^{211}At-MABG cell killing besides p53-p21 signaling. Thus, we aimed to investigate other important cell killing pathways. Here, we report the survival of neuroblastoma cell lines, and the preparation for RNA-seq of neuroblastoma cell lines.

We prepared two neuroblastoma cell lines. One is a neurob lastoma cell line, SK-N-SH, which has a wild-type p53 gene. The other is p53 mutated neuroblastoma cell line SK-N-BE(2C).[4] Cells were cultured in RPMI-1640 (Wako Pure Chemical Industries, Osaka, Japan) containing 10% Fetal Bovine Serum and 1% penicillin-streptomycin. The cells were incubated at 37°C in humidified air containing 5% CO_2 incubator. ^{211}At-MABG was synthesized, as previously described,[1] using ^{211}At produced at RIKEN or TIARA. The radioactivity of ^{211}At ($T_{1/2} = 7.2$ h) was measured from γ-rays emitted in ^{211}At decay using a high-purity germanium detector.

To examine RNA-sequencing at the same surviving rate, 10% and 80% survival, we investigate the survival of two neuroblastoma cell lines. Cells were incubated with 0, 0.1, 0.3, 1.0, 3.0, and 10.0 kBq/mL of ^{211}At-MABG for 24 hours. The cells were then washed with phosphate-buffered saline (PBS), suspended in growth medium, and seeded at 400 cells/well in a 96-well plate. After incubation for 14 days, the cells were incubated with 0.5 mg/mL of 3-(4,5-di- methylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) for 4 hours at 37°C. Absorbance at 590 nm was measured using a plate reader (VMax; Molecular Devices, Sunnyvale, CA). Rates of cell survival were normalized to the absorbance of control cultures treated with 0 kBq/mL. Figure 1 shows the survival curves of two neuroblastoma cell lines.

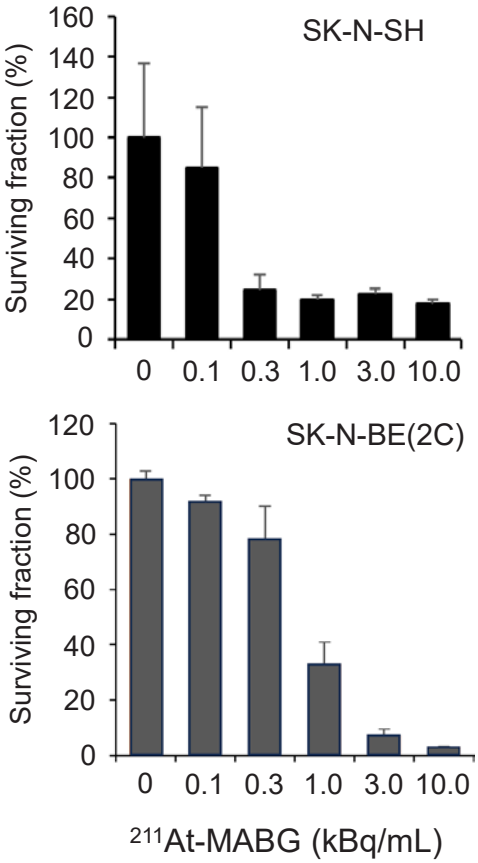

Fig. 1. Surviving fraction in % of two neuroblastoma cell lines, SK-N-SH (upper) and SK-N-BE(2C) (lower).

Cells were treated with ^{211}At-MABG at the dose of 80% and 10% survival, and samples of 5.0×10^5 cells was resuspended in TRIzol (Thermo Fisher Scientific, Waltham, MA, USA).

Here, we reported on the progress of our ongoing project to clarify the cell killing pathway of ^{211}At-MABG, which is not related to p53-p21 signaling. Futhermore, "^{211}At" was supplied from RIKEN via Supply Platform of Short-lived Radioisotopes, supported by JSPS Grant-in-Aid for Transformative Research Areas, Grant Number 22H04924. We are grateful to thank Dr. H. Haba, Dr. A. Nambu, and staff of RIKEN for ^{211}At production and transport.

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