

# RNA-seq reveals tumor radiation response and novel molecular targets on $\alpha$ -emitting meta-211At-astato-benzylguanidine therapy for malignant pheochromocytoma

**Purpose:** Targeted  $\alpha$ -particle therapy is a promising option for patients with malignant pheochromocytoma. Recent observations of *meta*-211At-astato-benzylguanidine (211At-MABG) in a pheochromocytoma mouse model showed a strong anti-tumor effect, but its molecular mechanism remains elusive (Y. Ohshima et al., 2018). Here, we showed the first comprehensive RNA-sequencing (RNA-seq) data of pheochromocytoma cells from *in vitro* 211At-MABG administration experiments, and screened key genes and pathways in the tumor  $\alpha$ -particle radiation response, in order to obtain novel molecular imaging and therapeutic targets.

**Methods:** We evaluated genome-wide transcriptional alterations of rat pheochromocytoma cell line (PC12) at 3, 6, 12 h after 211At-MABG treatment. In order to highlight 211At-MABG specific gene expression, we carried out the control experiment of  $^{60}\text{Co}$   $\gamma$

-rays irradiation. Ten-percent and eighty-percent iso-survival dose (0.8 and 0.1 kBq/ml for 211At-MABG, 10 and 1 Gy for  $^{60}\text{Co}$   $\gamma$ -rays) were used for the comparison of both treatments.

**Results:** Enrichment analysis of the differentially expressed genes (DEGs) and analysis of the gene expression profiles of the cell cycle checkpoints showed similar modes of cell death via p53-p21 signaling pathway following 211At-MABG treatment and  $\gamma$ -ray irradiation. Ten percent iso-survival dose of  $\gamma$ -ray irradiation and 211At-MABG showed cell cycle arrest at G2/M phase. Representative DEGs of 211At-MABG-treated cells between 80% and 10% survival showed the expression of key genes not only on the decrease in the survival, but also on the anti-therapeutic effects such as DNA repair, invasion, and metastasis. Furthermore, representative DEGs between  $\gamma$ -ray irradiation and 211At-MABG demonstrated that the expression of four potential genes including Otub1 related to ubiquitin mediated proteolysis was remarkably elevated only after treatment with 211At-MABG. Western blot analysis indicated the increase of translocator protein 18 kDa (TSPO) expression in 211At-MABG treated cells, suggesting the potential PET imaging probe.

**Conclusion:** Comprehensive RNA-seq revealed contrasting cellular responses to  $\gamma$ -ray irradiation and targeted  $\alpha$ -particle therapy leading to the identification of four novel potential genes (Mien1, Otub1, Vdac1 and Vegfa) for molecular imaging and therapeutic targets of 211At-MABG therapy. Moreover, our results suggest possible mechanism of the anti-tumor effect of 211At-MABG in pheochromocytoma.

**Reference:** Y. Ohshima, H. Sudo, S. Watanabe, K. Nagatsu, A.B. Tsuji, T. Sakashita, Y.M. Ito, K. Yoshinaga, T. Higashi, N.S. Ishioka, Antitumor effects of radionuclide treatment using  $\alpha$ -emitting *meta*-211At-astato-benzylguanidine in a PC12 pheochromocytoma model, *Eur. J. Nucl. Med. Mol. Imaging*, **45**, 999-1010. (2018)

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## Email Address

ohshima.yasuhiro@qst.go.jp

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**Primary author:** Dr OHSHIMA, Yasuhiro (National Institutes for Quantum and Radiological Science and

Technology)

**Co-authors:** Mr SASAKI, Ichiro (National Institutes for Quantum and Radiological Science and Technology); Dr ARAKAWA, Kazuharu (Keio University); Dr KONO, Nobuaki (Keio University); Dr ISHIOKA, Noriko (National Institutes for Quantum and Radiological Science and Technology); Dr WATANABE, Shigeki (National Institutes for Quantum and Radiological Science and Technology); Dr SAKASHITA, Tetsuya (National Institutes for Quantum and Radiological Science and Technology); Dr YOKOTA, Yuichiro (National Institutes for Quantum and Radiological Science and Technology)

**Presenter:** Dr OHSHIMA, Yasuhiro (National Institutes for Quantum and Radiological Science and Technology)