B-4

Simultaneous Inhibition of DNA Methylation and Histone De-acetylation for Enhanced SSTR2 Expression In Vitro

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BACKGROUND

Neuroendocrine tumor (NET) patients with diminished SSTR2 expression are not eligible for any type of SSTR2-specific imaging or treatment. Herein, we propose to epigenetically enhance and enable somatostatin receptor type 2 (SSTR2)-targeted theranostics for patients with NETs. Specifically, we have found that simultaneous inhibition of DNA methylation and histone de-acetylation enhanced SSTR2 expression and in vitro binding of [^{68}Ga]DOTATATE. Our **hypothesis** is that epigenetic modifiers with different mechanisms of action, have superior effect in upregulation of SSTR2 when comparing to the single drug treatment. Our approach will result in new targeted treatment strategies for patients who currently have very limited therapeutic options.

METHODS

To determine the anti-proliferative effects of **VPA, Decitabine** and the combination of both, all cell lines were treated for 72 hours and an MTT assay was used to determine the IC50. After a 48h incubation with subtoxic concentrations of either single drug or a combination, mRNA expression levels of SSTR2 were measured by quantitative real-time PCR. Following a 72h incubation with VPA, Decitabine, or a combination of both, cell lysates were collected, quantified, and Western blot analysis was performed to determine the effects of treatment on the protein expression of SSTR2. For functional SSTR2 analysis, DOTATATE was radiolabeled with 68Ga and incubated with cells at a concentration of 10 nM for 2 h. After washing, cells were lysed and radioactivity was assessed using a gamma counter. Activity was normalized to protein content via BCA assay and expressed as percent added dose per mg protein (%ID/mg).

RESULTS

We have shown that combination treatment with two epigenetic modifiers, both with different mechanisms of action, VPA (HDAC inhibitor) and Decitabine (DNMT inhibitor), had superior effect in upregulation of SSTR2 on mRNA, protein and functional levels when compared to the single drug treatment in BON, H727, and MZ cell lines. In contrast and most importantly, neither the fibroblast cell line WI-38 or the normal thyroid cell line Htori-3 showed an increase in [^{68}Ga]DOTATATE uptake after treatment.

CONCLUSIONS

We have revealed that combination treatment with HDAC (VPA) and DNMT (decitabine) inhibitors potentiated SSTR2 expression in NET cells and exhibited superior [^{68}Ga]DOTATATE binding comparing to either single drug. Furthermore, treatment of non-neuroendocrine cell lines exhibited no increase in radionuclide binding. The epigenetic upregulation of SSTR2 expression could improve the efficacy and toxicity profile for targeted radionuclide therapy of NETs with [^{177}Lu]DOTATATE.

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