Enhancing efficacy of PRRT for neuroendocrine tumors by combining with everolimus and histone deacetylase inhibitors

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Abstract

Background: Therapeutic options available for the treatment of Neuroendocrine tumors (NETs) include inhibitors of mTORC1; somatostatin analogs; and peptide receptor radionuclide therapy (PRRT) targeting the somatostatin subtype 2 receptor (SSTR2). While these approaches to NET treatment have improved outcomes for NET patients, efficacy is largely limited to disease stabilization and symptomatic relief – and complete responses are rare. Emerging biochemical evidence suggests that combinations of these drugs have the potential to improve outcomes for NET patients further. Within this context, we explored the potential for combining PRRT with everolimus, histone deacetylase inhibitors (HDACi), octreotide, and a MEK inhibitor (MEKi).

Methods: The effects on cell growth and clonogenic survival of BON-1 cells when incubated with everolimus, 4-phenylbutyric acid (4-PBA; HDACi), octreotide, and PD0325901 (MEKi) were evaluated (alone and in combination). The effect of the drugs on SSTR2 expression was measured by qRT-PCR (mRNA) and flow cytometry (protein). A [203Pb]DOTATOC SPECT/CT imaging study in BON-1 xenograft model was followed with single treatment of vorinostat (HDACi) or in combination with everolimus.

Results: The effects of everolimus were cytostatic, while treatments with MEKi and HDACi decreased both cell proliferation and clonogenic survival. Incubation of BON-1 cells with everolimus and HDACi upregulated SSTR2 by mRNA and protein. Combining everolimus with HDACi (as well as everolimus combined with MEKi) significantly decreased cell proliferation relative to these drugs alone as controls. Interestingly, the combination of everolimus and HDACi enhanced SSTR2 expression, while everolimus plus MEKi abrogated the upregulation of SSTR2 induced by treatment with everolimus alone. [203Pb]DOTATOC SPECT/CT imaging confirmed the potential to enhance radiolabeled peptide uptake in BON-1 xenograft tumors by pretreatment with the combination of everolimus and HDACi.

Conclusion: This study suggests the co-treatment of everolimus and HDACi can be used to enhance the therapeutic efficacy of PRRT by upregulating SSTR2 and increasing anti-tumor effects for NETs.
Neuroendocrine tumors (NETs) are rare, but its incidence is on the rise.\textsuperscript{1} In the development of effective therapy, high expression of somatostatin subtype 2 receptors (SSTR2) is recognized as a target for drug delivery, including peptide receptor radionuclide therapy (PRRT) using $^{\text{DOTA}^{\circ}}$-Tyr$^{3}$-octreotide (DOTATOC; Fig. 1). While the most of NETs has been known to highly express SSTR2, the SSTR2 expressions in tumor environment are heterogeneous and down-regulated in many high-grade NETs (Fig. 2), which correlates with the poor survival.\textsuperscript{2} Recent studies suggested that the therapeutic efficacy of PRRT can be potentially enhanced with histone deacetylase inhibitor (HDACi) by epigenetically upregulating SSTR2 and sensitizing the tumors to radiations.\textsuperscript{3, 4}

Besides the PRRT, there are several other therapeutic options for NETs. One promising approach is targeting mammalian target of rapamycin (mTOR). Everolimus, an inhibitor mTOR, has significantly improved the progression-free survival (PFS) for the advanced NETs.\textsuperscript{5} Inhibition of MAPK pathway in combination with Everolimus has been proposed for treatment of NETs based on the observation that mTOR inhibition can activate the MAPK pathway through a PI3K dependent feedback loop.\textsuperscript{6} Somatostatin analogs (i.e. Octreotide, Lanreotide) have been also used for NET patients as a single treatment or as a combination with other therapeutics. Despite these efforts in the development of effective therapy, combination of the drugs with PRRT or the effect of previous therapeutic regimes on following PRRT has not been extensively explored. In this context, we explored Everolimus, HDAC inhibitors, Octreotide, and MEK inhibitor to test if combining the drugs with PRRT might have a potential to increase the therapeutic efficacy of PRRT.
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Everolimus, the MEK inhibitor, and 4-PBA inhibit BON-1 cell proliferation significantly.

The MEK inhibitor decreases the clonogenic survivals of BON-1 cells in dose dependent manner while everolimus works cytostatic.

Fig. 3. Cell growth of BON-1 cells treated to 100 nM Everolimus (Ever), 2 mM 4-Phenylbutyric acid (4-PBA), 10 nM PD0325901 (MEK inhibitor; MEKi), and 100 nM Octreotide (Oct). 50,000 cells were seeded to 6-well plates, and after 2 days, the cells were treated to the drugs and the cell numbers were counted by the Coulter Counter at 2, 4, and 6-day post-treatments. * p<0.05

Fig. 4. Clonogenic survivals of BON-1 after 4-day treatments of the drugs under investigation in increasing concentrations. 15,000 cells were seeded to 24-well plates and after 2 days, the cells were treated to the drugs for 4 days. 5 μM of DHE was incubated with the cells for 30 min in the incubator and the oxidation state was analyzed by the flow cytometry. Results are normalized to the untreated control and represented as normalized mean ± SD; * p<0.05; ** p<0.01; *** p<0.005, # p<0.001 (n = 6 from two biological replicates).

Fig. 5. DHE oxidation in BON-1 cells after 4 day treatment of the drugs investigated. 1.0 x 10⁶ BON-1 cells were seeded into 6-well plates and after 2 days of incubation, the cells were treated to the drugs for 4 days. 5 μM of DHE was incubated with the cells for 30 min in the incubator and the oxidation state was analyzed by the flow cytometry. Results are normalized to the untreated control and represented as normalized mean ± SD; * p<0.05; *** p<0.005; ### p<0.0001 (n = 6 from two biological replicates).
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- Everolimus, MEK inhibitors, and 4-PBA significantly inhibit BON-1 cell proliferation at various degrees.
- Everolimus and HDAC inhibitors (e.g., 4-PBA) upregulate SSTR2 by mRNA and protein.
- The dual combination of everolimus and 4-PBA further inhibits cell proliferation and upregulates SSTR2.

Data suggests the co-treatment of everolimus and HDACi can enhance the efficacy of PRRT by the mechanisms involving receptor upregulation and increased anti-tumor effects.

Key parameters (i.e., dose, time) are required to be determined for the ideal pharmacological enhancement of SSTR2 in vivo.

References