Background

Neuroendocrine tumors (NETs) comprise a very heterogeneous group of malignancies, as they can arise from neuroendocrine cells in virtually every internal organ. The management of localized disease involves surgical resection with curative intent. To ensure complete removal of the tumor, currently often unnecessary radical resections are performed, and this is associated with substantial morbidity. Furthermore, residual disease often leads to recurrence of these lesions. Therefore, techniques that lead to more specific and less invasive ablation of tumor cells are warranted, and targeted photodynamic therapy is an interesting treatment option. Here, a photosensitizer is delivered to the tumor by coupling it to a molecule that targets a receptor that is specifically (over)expressed on the tumor cells. Upon activation with near infrared light, the photosensitizer induces production of the toxic singlet oxygen and reactive oxygen species, thereby causing cell death. Two G protein-coupled receptors that are regularly overexpressed on neuroendocrine tumors are the somatostatin receptor (SSTR), and the glucagon-like peptide 1 receptor (GLP1R). In the current project, we developed and characterized peptide-photosensitizer conjugates for SSTR- and GLP1R-targeted photodynamic therapy.

Materials and methods

Zinc-phthalocyanine based photosensitizers TT1-maleimide and NETCure2-maleimide were conjugated to a sulfhydryl group on the primary amine of Lys40 in GLP1R-targeting peptide exendin-4, and of dPhe1 in SSTR-targeting peptide octreotide. Properties such as absorbance and fluorescence of all conjugates were characterized in dimethyl sulfoxide (DMSO), phosphate buffered saline (PBS) and PBS with bovine serum albumin (PBS-BSA). To determine half maximal inhibitory concentrations (IC_{50}), chinese hamster lung cells stably transfected with GLP-1R (CHL-GLP1R) or HCT116 cells stably transfected with SSTR (HCT116-SSTR2) were co-incubated with either exendin-4-conjugates and ^{111}In-labeled exendin-4-DTPA as a competitor or octreotide-conjugates and ^{111}In-labeled octreotide-DTPA as a competitor, respectively. Then, light-induced toxicity of the CHL-GLP1R cells, cells with physiological receptor expression (INS) or without receptor expression (PANC) by exendin-4-conjugates was determined. Cells were incubated for 4 hours with various concentrations of the conjugates and after washing away the unbound fraction, cells were illuminated with 690 nm light using a light emitting diode (LED 150 J/cm^2 at 200 mW/cm^2). Non-illuminated cells served as controls. Finally, BALB/c nude mice carrying subcutaneous xenografts of CHL-GLP1R tumors were injected with 20 µg exendin-4-TT1, and an ex vivo fluorescent biodistribution to determine uptake in tumors and other tissues (liver, spleen, kidneys, blood, pancreas) was performed at 4 and 18 hours post-injection.

Results

Spectral properties of TT1 and NETCure2 were retained after conjugation to both peptides, with excitation and emission maxima of 682 nm and 696 nm in DMSO. Dissolving the conjugates in PBS led to a blue-shift and a decreased intensity of the Q-band, and this could partly be rescued by adding bovine serum albumin. All conjugates bound to target-expressing cells. Cytotoxic effect of exendin-4-conjugates was evaluated and exendin-4-TT1 induced concentration dependent cell death of both INS and CHL-GLP1R cells upon illumination, while exendin-NETCure2 induced cell death of only CHL-GLP1R. No cytotoxic effects on PANC cells were observed for both conjugates. Upon injection in BALB/c nude mice carrying subcutaneous CHL-GLP1R xenografts, relative tumor uptake of 1.46±0.52 %ID/g and

3.24±1.05 was found for exendin-4-TT1 at 4 and 18 hours post injection, respectively. Non-target specific uptake of exendin-4-TT1 was mainly found in the liver (37.98±3.69 %ID/g at 18 hours post injection) and spleen (56.04±13.36 %ID/g at 18 hours post injection).

Conclusions

In conclusion, the novel octreotide- and exendin-photosensitizer conjugates show specific binding to target-expressing cells in vitro. Exendin-4-TT1 induces cell death upon illumination with high potency, and it homes to GLP-1R expressing tumors in vivo. In ongoing experiments, we are determining in vitro efficacy of the octreotide-photosensitizer conjugates, and we will ultimately determine in vivo biodistribution and light induced ablation of target-expressing tumor cells in vivo by all conjugates.

Lay abstract

Background

Currently, the only option to cure patients with neuro-endocrine tumors is complete surgical removal of the tumor. In this project, we aim to develop molecules that can aid the surgeon to remove all tumor cells using an approach called tumor targeted photodynamic therapy. In this therapy, a molecule called a photosensitizer is brought to the tumor cells, and activated with near infrared light. Upon activation, the photosensitizer produces reactive oxygen species (ROS). These ROS are very toxic to the cells and thus induce cell death. In the current project, we want to develop efficient and stable photosensitizers and couple them to molecules that bind specifically to neuroendocrine tumor cells, named exendin-4 and octreotide. We will test the ability of these coupled molecules to eliminate neuroendocrine tumor cells in both cell cultures and in animal models of neuroendocrine tumors.

Results

We have successfully designed and produced two photosensitizers named TT1 and NETCure2. We have added a functional group that enables coupling of these photosensitizers to the molecules that specifically bind to neuroendocrine tumor cells, exendin-4 and octreotide. All coupled molecules were shown to bind to cells in culture, and especially exendin-4-TT1 was very potent in inducing cell death upon illumination. Importantly, this exendin-4-TT1 also traveled to the tumor in an animal model. Currently, we are investigating the potency of the octreotide-photosensitizers in cell cultures, and ultimately we will test all molecules for targeting the tumor in animal models, and by inducing cell death in these tumors upon illumination.