Sequence defined cMET/HGFR-targeted polymers as gene delivery vehicles for the theranostic sodium iodide symporter (NIS) gene

Sarah Urmauer1, Stephan Morzy2, Ana Khac Levacic2, Andrea M. Müller1, Christina Schug1, Kathrin A. Schmohl1, Nathalie Schwenk1, Christian Zeib1, Janette Carlsson1, Peter Bartenstein2, Ernst Wagner1, Christine Spitzweg1

1Department of Internal Medicine II, 2Department of Pharmacy, Center of Drug Research, Pharmaceutical Biotechnology, 3Department of Nuclear Medicine, LMU Munich, Germany

Introduction
The sodium iodide symporter (NIS) in its role as well characterized reporter and therapy gene represents an outstanding tool to target different cancer types allowing non-invasive imaging of functional NIS expression by 131I-spectroscopy and therapeutic application of 131I. Based on its overexpression on the surface of the vast majority of cancer types, the cMET/Hepatocyte growth factor receptor (HGFR) serves as an ideal target for tumor-selective gene delivery.

Materials and Methods
In the current study, we used sequence defined polymers as non-viral gene delivery vehicles comprising polyethylene glycol (PEG) and cationic (oligoethanolamin) amide cores coupled with a cMET-binding-peptide (cMBP2) to target the cMET/HGFR-receptor in a human hepatocellular cancer (HuH7) mouse model. These polymers were complexed with human NIS-DNA (polyplexes) and tested for receptor-specificity, transduction efficiency and therapeutic efficacy.

Results

Fig. 1 In vitro iodide uptake studies in HCC cells (HuH7) with high cMET/HGFR expression levels demonstrated high transduction efficiency and cMET-specificity of NIS-encoding DNA polyplexes coupled with cMBP2 (cMBP2-PEG-Stp/NIS) compared to polyplexes without ligand (Ala-PEG-Stp/NIS) and polyplexes containing non-coding DNA (cMBP2-PEG-Stp/Antisense-NIS) (A). Pretreatment with the NIS-specific inhibitor perchlorate led to reduced transduction efficiency. To verify dependency on cMET/HGFR expression levels, the HCC cell line Hep3B with low expression levels was used as control, proving strong correlation between receptor and transduction levels (B, C).

Fig. 2 Tumor recruitment and vector biodistribution were investigated in vivo by 123I-spectroscopy showing high tumor-selective iodide accumulation in cMBP2-PEG-Stp/NIS-treated mice (6.8 ± 1.6% IDg/123I, biological half-life 3 h) 48h after intravenous polyplex delivery, while injection of control vectors Ala-PEG-Stp/NIS and cMBP2-PEG-Stp/Antisense-NIS did not result in specific iodide uptake. A subset of cMBP2-PEG-Stp/NIS-treated mice was pretreated with the NIS-specific inhibitor perchlorate 30 minutes before 123I application, to demonstrate NIS-dependency of tumoral iodide uptake.

Fig. 3 Therapy studies with 3 cycles of polyplexes and 131I applications resulted in a significant delay in tumor growth (A) and prolonged survival (B) of cMBP2-PEG-Stp/NIS-treated mice.

Fig. 4 Immunofluorescence analysis of frozen tumor sections after treatment exhibited reduced cell proliferation and blood vessel density in tumors of animals treated with cMBP2-PEG-Stp/NIS that received 131I as compared to all control groups. To determine cell proliferation sections were stained with a Ki67-specific antibody (green) and to label blood vessels an antibody against CD31 (red) was used.

Conclusions
In conclusion, our data demonstrate the enormous potential of cMET-targeted sequence defined polymers combined with the unique theranostic function of NIS allowing for optimized transfection efficiency while eliminating adverse effects such as toxicity or high immunogenicity.

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