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

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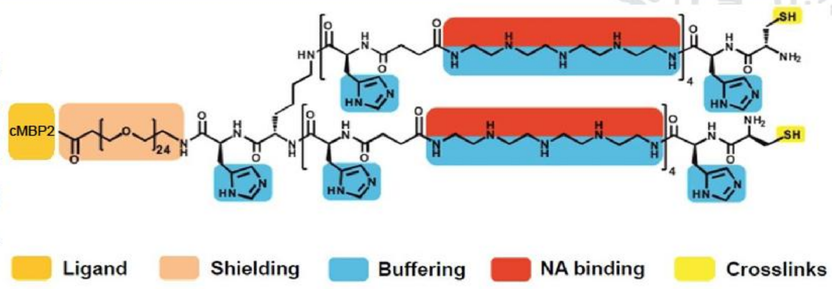
Introduction

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 ^{123}I-scintigraphy and therapeutic application of ^{131}I. 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 (oligoethanoamino) amide cores coupled with a cMET-binding-peptide (cMBP2) to target the cMET/HGF-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).

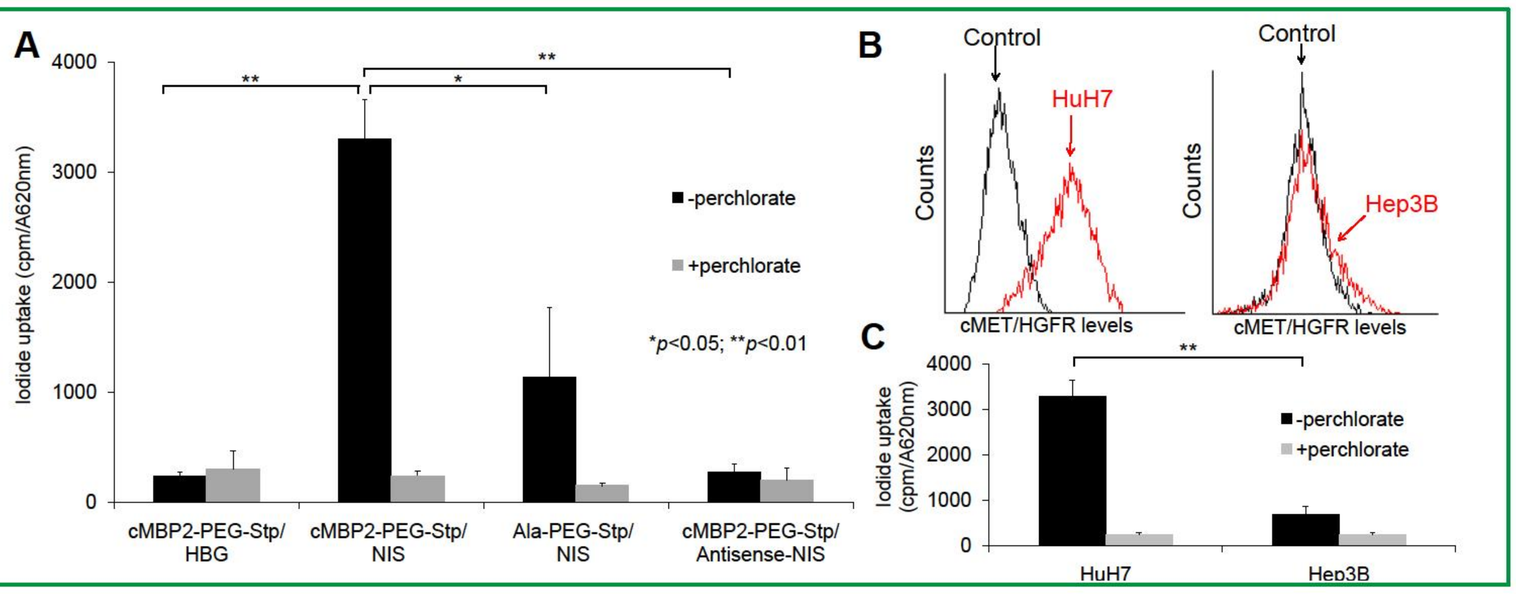
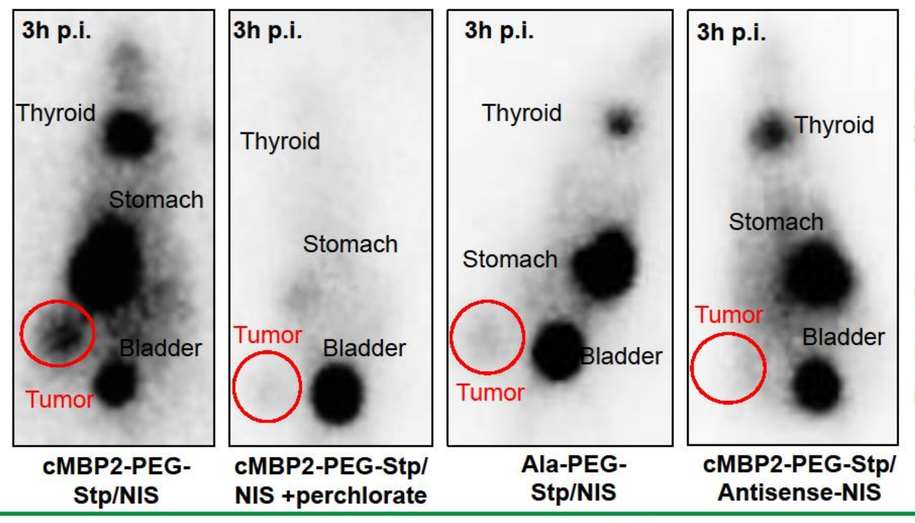


Fig. 2 Tumor recruitment and vector biodistribution were investigated *in vivo* by ^{123}I-scintigraphy showing high tumor-selective iodide accumulation in cMBP2-PEG-Stp/NIS-treated mice (6.6±1.6% ID/g ^{123}I, biological half-life 3 h) 48 h after intravenous polyplex application, while injection of 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 min before ^{123}I application, to demonstrate NIS-dependency of tumoral iodide uptake.



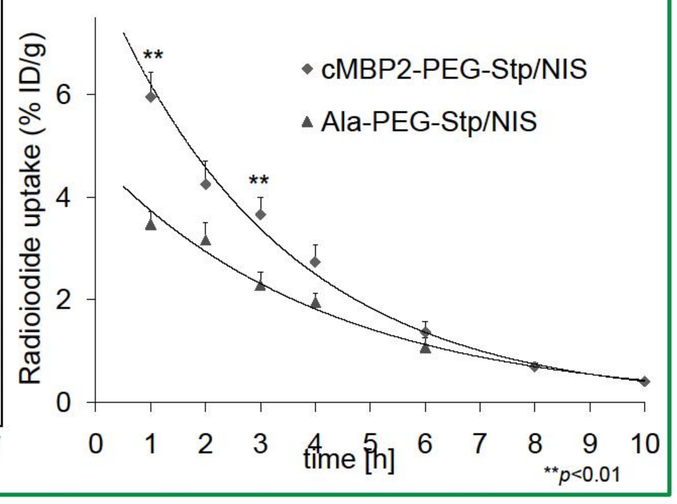


Fig. 3 Therapy studies with 3 cycles of polyplexes and ^{131}I applications resulted in a significant delay in tumor growth (A) and prolonged survival (B) of cMBP2-PEG-Stp/NIS-treated mice.

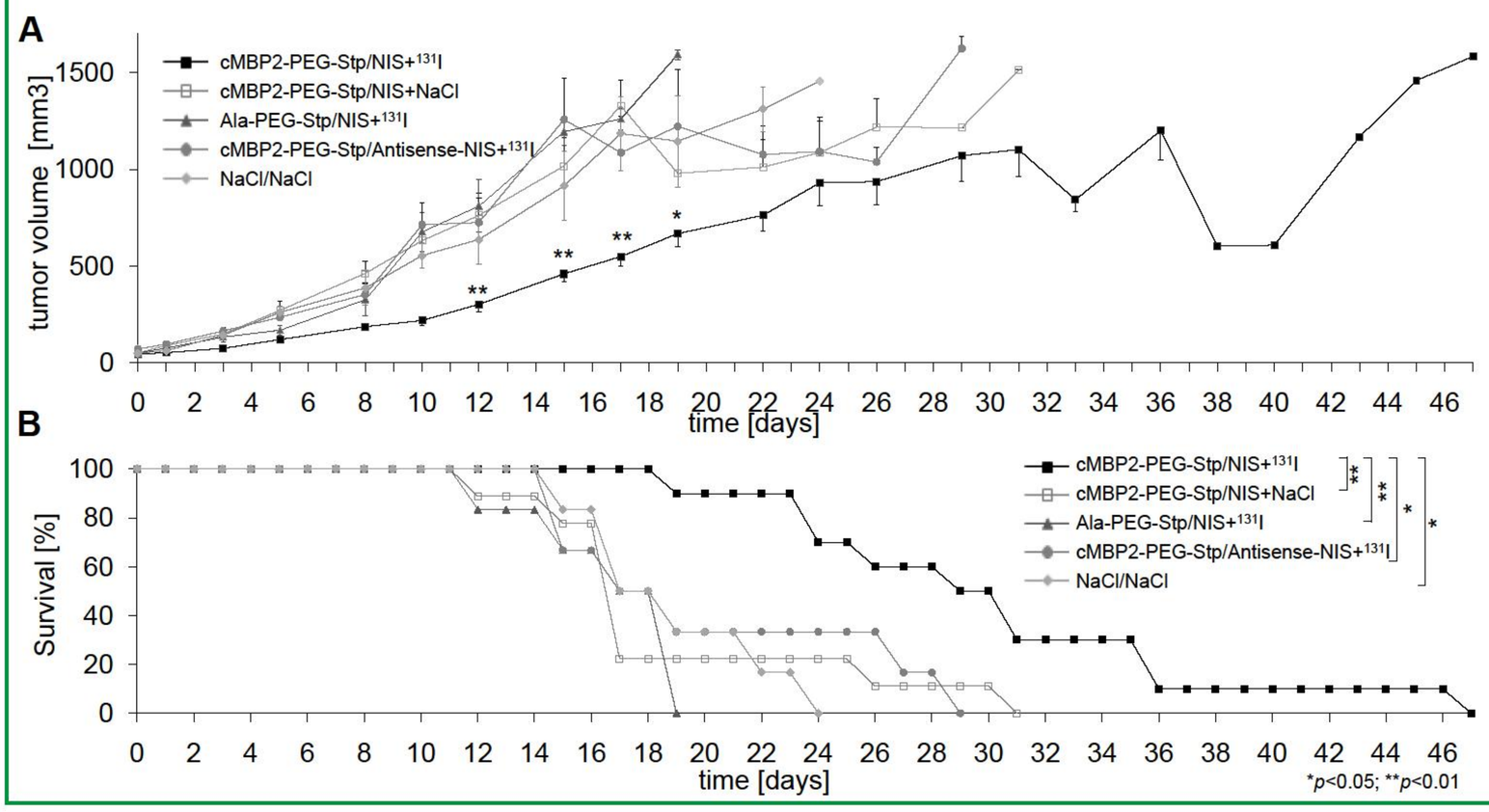
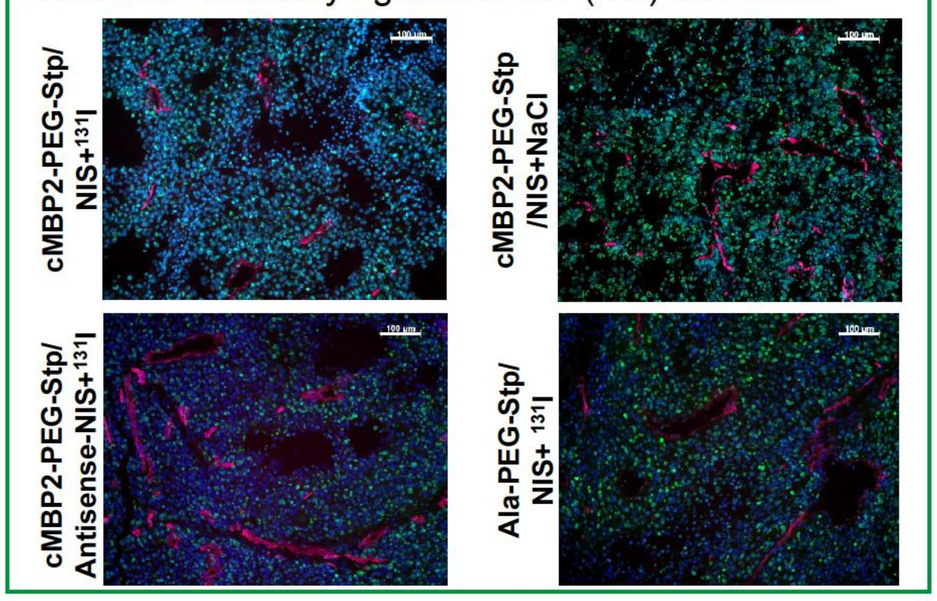


Fig. 4 Immunofluorescence analysis of frozen tumor sections after treatment exhibited reduced cell proliferation and blood vessel density in tumors of animals treated with cMBP2-PEG-Stp/NIS that received ^{131}I as compared to all control groups. To determine cell proliferation sections were stained 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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