Nanobioconjugates for Differential Imaging of Solid and Metastatic Brain Tumors

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ABSTRACT SUMMARY

Dynamic contrast enhancement is shown to allow differential MRI detection of human primary brain and metastatic breast cancers in the brain applied to xenogeneic mouse models. Gadolinium-DOTA contrast reagents were covalently attached to polymalic acid (PMLA) nanobiopolymer together with cancer-specific monoclonal antibodies (mAb) previously shown to target primary brain and breast cancer1-3. After IV tail injection of tumor-type specific PolycefinTM contrast reagent, enhanced T1 contrast in targeted tumor was sustained for prolonged times, while that in healthy brain or non-targeted tumor was washed out. Differential imaging was confirmed using fluorescence Xenogen imaging. The retention of the specific reagent in the tumor, underlying the mechanism of differential imaging, was demonstrated by confocal microscopy.

INTRODUCTION

A significant clinical problem with brain metastatic tumors is drug delivery and lack of a specific MRI response identifying the type of cancer at the starting point for optimal treatment. A MRI response in a patient’s brain may result from infection following a preceding chemotherapy, from metastasis of primary lung/breast cancer or from a new primary brain tumor. Unlike lung/breast, brain biopsy is often technically impossible. Here we present an approach to overcome these problems.

EXPERIMENTAL METHODS

For generating orthotropic metastatic brain cancers, BT-474 cells for HER-2 positive breast cancer, MDA-MB-468 cells for Triple Negative Breast Cancer (TNBC), and U87MG cells for glioblastoma were injected into the brain of mice. After growth, good MRI contrast was acquired with 0.1 mmol/kg of Multihance®, a clinical contrast reagent. For differential imaging with PolycefinTM contrast reagents, the injected amounts were identical and referred to the same value of relaxation T1 as used for Multihance®. Tumor specificity of PolycefinTM contrast reagents was acquired by two types of mAbs attached simultaneously to the polymer (Fig. 1): anti-mouse TfR mAb for extravasation (transcytosis of cancer vessel endothelium) and either Herceptin (targeting HER-2) or Cetuximab (targeting EGFR on TNBC and glioblastoma). Fluorescence labeling with Alexa Fluor 680 was optional for Xenogen IVIS 200 live animal imaging system and confocal microscopy. The synthesis is depicted in Fig. 2. Composition of the contrast reagents in terms of malic acid, mAbs, and gadolinium were quantified post-synthetically. An average amount of 70 attached gadolinium-DOTA per molecule of contrast reagent was synthesized. Relaxivity was 140 mM⁻¹s⁻¹ per molecule of contrast reagent, the hydrodynamic diameter measured by DLS was 16±2 nm, and of zetapotential -7.0 mV to -8.7 mV, depending on the kind of mAb. For MRI, usually 150 µL solution of contrast reagent having T1 relaxation of 15±1 msec was IV injected via tail. MRI was acquired at Core facility in the Biomedical Imaging Research Institute using a micro MRI 9.4-Tesla Bruker, 94/20 BioSpec MRI system.

RESULTS AND DISCUSSION

We used the nanobiopolymer platform, PMLA, for synthesis of the contrast reagents to achieve differential brain tumor imaging (Fig. 1). To be independent of EPR-effect, extravasation into cancer through transcytosis was targeted by anti-mouse TfR mAb. Specific targeting to cancer cells overexpressing HER-2 or EGFR was achieved by Herceptin and Cetuximab respectively. The contrast reagents were small and nonspherical, not carrying PEG, thus favoring diffusion and moderate circulation times before being washed out from the blood circulation.
When contrast reagent Multihance® was used, acceptable contrast of tumor was seen at 20 minutes after injection and before the reagent was washed out. At 1 hour post injection, tumors were no longer detectable. In contrast, EGFR targeting Polycefin™ contrast reagent, as an example in Fig. 3, showed maximum MRI enhancement of tumor at 45-60 min after injection that remained high for at least 3 hrs.

Similar results were obtained for imaging of glioblastoma using the same reagent, or for HER-2 positive tumor in the presence of HER-2 targeting Polycefin™ contrast reagent. However, when HER-2 targeting Polycefin™ contrast reagent was used in the presence of TNBC in Fig. 3 insignificant enhancement was noted that disappeared after 1 hr. In contrast, when HER-2 positive tumor was imaged with HER-2 targeting Polycefin™ contrast reagent, differential imaging was seen. This is demonstrated in Fig. 4, when we implanted metastatic HER-2 positive (right hemisphere) and TNBC (left hemisphere) side by side in the same brain. Imaging with Multihance® shows both tumors present, each one in either hemisphere. We demonstrated that HER-2 Positive metastatic breast cancer was detected using HER-2 targeting contrast reagent, while TNBC or glioblastoma could not be visualized. These examples convincingly demonstrated that different types of tumor in the same brain could be identified side by side, a fact relevant after translation into clinics. Independent confirmation of differential imaging was obtained by Xenogen imaging of brain after injection of fluorescence labeled contrast reagents that showed high degree of fluorescence exclusively only in the targeted tumors.

Confocal microscopy of cryostat sections from TNBC after MRI with EGFR targeting Polycefin™ contrast reagent confirmed that fluorescent labeled reagent was contained in the brain that had passed the brain-tumor barrier (BTB) and accumulated in tumor cells. This result validated the underlying assumption that differential imaging is achieved by tumor specific targeting and uptake.

CONCLUSION

We have developed a system for differential imaging of primary and metastatic brain tumors based on tumor-type specific targeting and dynamic contrast enhancement using MRI. It is assumed that with minor modifications the method can be translated into clinics.

REFERENCES


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