A novel membrane-permeant contrast agent for ultra-sensitive brain tumor detection by MRI

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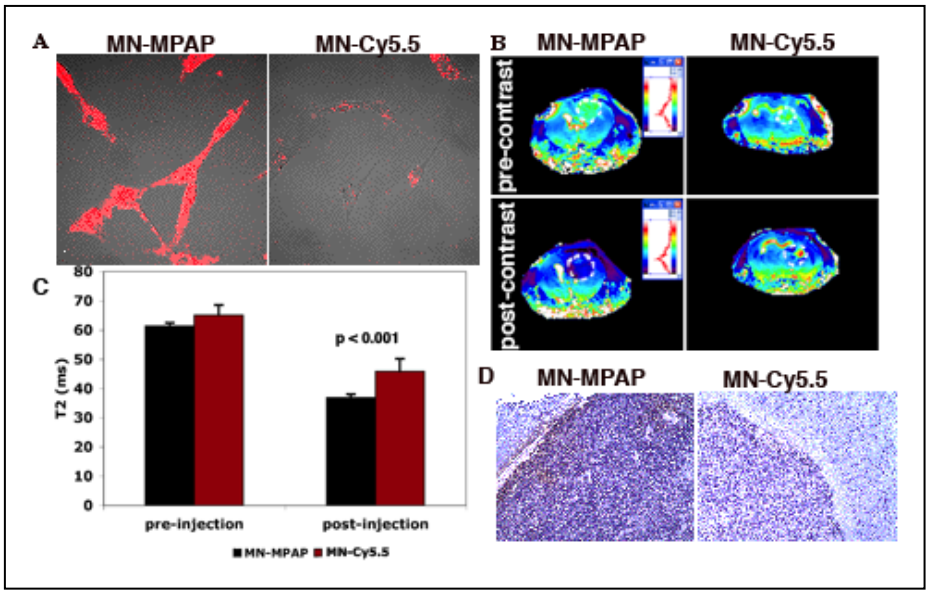
Introduction: One of the key challenges hindering effective therapy against brain cancer is defined by the inability to detect brain tumors at an early stage. Furthermore, the rapid growth and relative lethality of brain cancer predicate the vital importance of close monitoring the development of the pathology and its response to therapeutic intervention. With this in mind, we designed a novel membrane-permeant contrast agent, MN-MPAP, which consists of a superparamagnetic iron oxide nanoparticles, for MR imaging, labeled with the near-infrared dye Cy5.5, for near-infrared optical imaging, and conjugated to myristoylated polyarginine peptides (MPAP), as a membrane translocation module. We have previously demonstrated the remarkable capacity of MPAP to translocate cargo across cellular membranes and other biological barriers, including the blood-brain barrier (1).

Materials and methods: *Synthesis:* MPAP was synthesized by conventional Fmoc solid phase chemistry. The final product of C_{14}-β-Ala-$(Arg)_7$-Cys-NH_2 gave optimum yield and the final MPAP-Cys was characterized by HPLC and Mass spectroscopy. MN-Cy5.5 was synthesized as described before (2). MPAP was conjugated to MN-Cy5.5 through its Cys side chain sulfur group. *In vitro studies:* The cellular uptake of MN-Cy5.5-MPAP or MN-Cy5.5 by U-87 human glioblastoma cells was determined using a fluorescence assay on cell lysates, flow cytometry, and iron uptake assay. The subcellular distribution of the probes was assessed by confocal microscopy. *In vivo studies:* In vivo MR imaging was performed on mice bearing orthotopic U-87 glioblastomas before and 24 hrs after i.v. injection of MN-MPAP. Imaging was performed on a 9.4 T Bruker horizontal bore scanner (Billerica, MA) equipped with Para Vision 3.0 software. The imaging protocol consisted of coronal and transverse T2 weighted spin echo (SE) pulse sequences. To produce T2 maps, the following imaging parameters were used: SE TR/TE = 3000/ 8,16,24,32,40,48, 56, 64; FoV = 19.2 X 19.2 mm; matrix size 128 X 128; slice thickness = 0.5 mm; resolution 312 X 312 μm; and imaging time of 4.0 min and 16 sec.

Results: In a quantitative cell-binding assay on cell lysates, U-87 glioblastoma cells displayed a significantly higher uptake of MN-Cy5.5-MPAP than the control MN-Cy5.5 probe ($p < 0.05$). Flow cytometry revealed a remarkable 30-fold higher Cy5.5 fluorescence per cell, following treatment with MN-Cy5.5-MPAP, as compared with MN-Cy5.5. These findings were closely mirrored by measurements of cellular iron uptake. On confocal microscopy, cells incubated with MN-Cy5.5-MPAP were associated with abundant staining over the area of the entire cell inclusing the cytosol, whereas cells incubated with MN-Cy5.5 displayed only spotty, primarily endosomal fluorescence (Fig. 1A). In vivo MRI established the value of MN-Cy5.5-MPAP for tumor localization and delineation (Fig. 1B). T2 map analysis demonstrated higher accumulation of MN-Cy5.5-MPAP in tumors than MN-Cy5.5, as reflected by the change in tumoral T2 relaxation times following injection of the contrast agent (Fig. 1C). Final validation of the considerable tumoral accumulation of MN-Cy5.5-MPAP was obtained by histology on brain tissue (Fig. 1D, DAB-enhanced Prussian Blue stain for iron).



Summary: The noninvasive detection of brain tumors when they are still small represents a formidable challenge from an imaging standpoint. Here, we describe the development and testing of a contrast agent for improved detection of brain tumors. Our findings suggest that this agent mediates its effects by enhancing the translocation of the MN label across the leaky tumor vasculature and into the tumor cell. The development and application of this novel construct for neuroimaging have important clinical implications, since MR is a clinically acceptable modality.

References:

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