Peptide-based MRI contrast agent and near-infrared ﬂuorescent probe for intratumoral legumain detection

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Abstract

In this study, we developed a MRI contrast agent ([Gd-NBCB-TTDA-Leg(L)]) and a NIR fluorescence (NIRF) probe (CyTE777-Leg(L)-CyTE807) to monitor legumain activity in tumors. Peptide cleavage of the MRI contrast agent by legumain can promote contrast enhancements. The cleavage effect on NIR probes relieves the self-quench of the probe. *In vivo* MR images showed that [Gd-NBCB-TTDA-Leg(L)] attained 55.3 fold higher enhancement. Similarly, CyTE777-Leg(L)-CyTE807 attained 15.2 fold higher enhancement. These data indicate that [Gd-NBCB-TTDA-Leg(L)] and CyTE777-Leg(L)-CyTE807 may be promising tools to image the legumain-expressing cancers for diagnoses.

Introduction

Many studies have clearly revealed the correlation between protease and poor prognosis in patients with cancer [1], indicating that proteases may be as markers to predict tumor recurrence. Recent studies have shown that legumain is abundantly expressed in human solid tumors [2]. Therefore, development of imaging probes for detecting legumain activity is likely to aid in tumor diagnosis. Herein, we report a MRI contrast agent and a NIR fluorescence probe for legumain over-expressing tumor detection.

Materials and Methods

For the *in vivo* MR images, nude mice bearing CT-26 tumors were studied by MR imaging. The MR imaging of mice was performed at pre-injection and various time points (2 and 24 h) post injection. Optical imaging was acquired at pre-injection and 24 h post-injection using an IVIS system (ex / em = 745 nm / 820 nm).

Results and Discussion

As shown in Figures (A) and (B), systematic representation of [Gd-NBCB-TTDA-Leg(L)] and CyTE777-Leg(L)-CyTE807 for legumain detection. *In vivo* MR imaging was carried out for [Gd-NBCB-TTDA-Leg(L)] and the control contrast agent ([Gd-NBCB-TTDA-Leg(D)]). As shown in Figures 1 (C) and (D), the contrast enhancements of [Gd-NBCB-TTDA-Leg(L)] reached 254.2% and 3.9% at 2 h and 24 h, respectively. In contrast, only slight enhancements (4.6% at 2 h, and 3.3% at 24 h) were observed for control contrast agent. To investigate the usefulness of CyTE777-Leg(L)-CyTE807, *in vivo* imaging of mice bearing CT-26 tumors was attempted. As shown in Figures (E) and (F), high fluorescent intensities were observed by treated with NIRF probe (3.34×10^9 photons/min at 24 h). On the contrary, minimal signal was found by treated with CyTE777-Leg(D)-CyTE807 (0.22×10^9 photons/min at 24 h). The relative fluorescent enhancement was 15.2 fold higher with CyTE777-Leg(L)-CyTE807 injection.

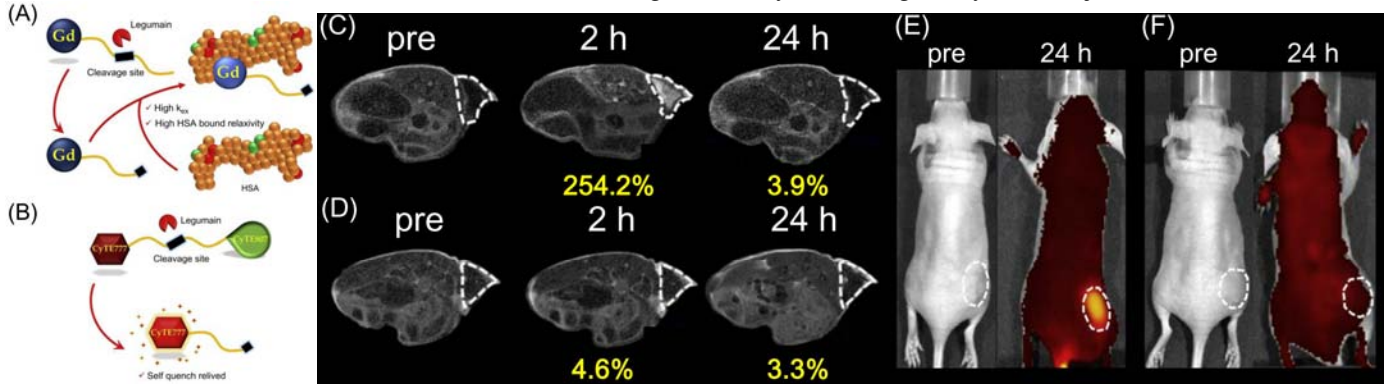

Figure 1. Systematic representation of (A) MRI contrast agent and (B) NIR fluorescent probe; *in vivo* MR images of mice bearing CT-26 xenografts pre- and post-injection of [Gd-NBCB-TTDA-Leg(L)] (C) and control contrast agent ([Gd-NBCB-TTDA-Leg(D)]) (D); *in vivo* NIR fluorescence images of CyTE777-Leg(L)-CyTE807 (E) and the control probe (CyTE777-Leg(D)-CyTE807) (F).

Conclusion

In summary, a MRI contrast agent and a NIR fluorescent probe were successfully developed. Legumain-mediated cleavage of the substrate peptide increases contrast enhancements or fluorescent intensity. Our data indicate that the contrast agent and the optical probe can specifically and efficiently target legumain-expressing cancers *in vivo*. This unique development may useful for detection of legumain expression in biomedical studies.

References

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