A Peptide Based Dual-Labeled Agent Targeted to Interleukin 11 Receptor Alpha-Chain for Molecular Imaging

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Abstract

This study describes the synthesis and biologic evaluation of a IL11 peptide targeted dual-labeled imaging agent as a potential probe for detecting human breast cancer with magnetic resonance imaging (MRI) and optical imaging. The IL11 peptide substrate was synthesized and conjugated with the TTTDA chelating moiety and Cy5.5 (Gd-NB-TTTDA-IL11-cy5.5) via solid-phase peptide synthesis, purified by reversed-phase HPLC and identified by mass. The T1 relaxivity of Gd-NB-TTTDA-IL11 was 5.48 mM⁻¹ s⁻¹ at 37 ± 0.1°C and 0.47 T. The results of in vitro optical and MR imaging indicated that the targeted contrast agent specifically targeted to MDA-MB231 human breast cancer cell and lead to significant enhancement (57%). The preliminary results also demonstrated that the bimodal IL11 peptide analog could be used to detect IL-11Rα positive breast cancer with MRI and fluorescence microscope at the cellular level.

Introduction

Magnetic resonance imaging has become the leading tool for imaging fine details of anatomy as well as physiology. Optical imaging is providing a sensitive and specific method for the detection and localization of the biochemical appearance in vivo. Interleukin-11 (IL-11) is a pleiotropic cytokine, play an important role in the bone metastasis of human breast cancer. Breast cancer cells express IL-11 receptor (IL-11Rα) and secrete IL-11, which in turn have been shown to stimulate osteoclasts. Thus, IL-11Rα provides highly selective sites that differentiate tumor cells from normal cells. The cyclic peptide c(CGRRAGGSC)(IL11 peptide substrate) which is known to target IL-11Rα was synthesized and characterized. In this study, we designed and synthesized a dual-labeled imaging agent (Gd-NB-TTTDA-IL11-cy5.5) which is target to IL-11Rα upon this IL11 peptide substrate. Finally, cell cytotoxicity, in vitro MR and optical imaging were performed.

Methods

IL11 peptide substrate was synthesized and conjugated with the TTTDA chelating moiety and Cy5.5 (Gd-NB-TTTDA-IL11-cy5.5) via solid-phase peptide synthesis, purified by reversed-phase HPLC and identified by mass. Gd-NB-TTTDA-IL11-cy5.5 were added at the predetermined concentrations (1 μM) the cells were washed three times with PBS buffer solution. In addition, the nucleus was stained with 4′,6-diamidino-2-phenylindole solution(200 nM) for 3 minutes followed by washing with PBS buffer solution three times and the cells were inspected using fluorescence microscope. In vitro studies, MDA-MB-231 and HT-1080 cell lines were incubated with Gd-NB-TTTDA-IL11 washed by PBS buffer solution and scanned by 3.0 T MRI.

Results and Discussion

Gd-NB-TTTDA-IL11-cy5.5 was synthesized and characterized. In vitro specific targeting was investigated using fluorescence microscope as shown in Figure 1. It is well known that IL-11Rα undergoes receptor-mediated endocytosis, which results in accumulation of the ligand–receptor complex inside the cells. In the other hand, in vitro fluorescence imaging can exhibit detailed microscopic information at the subcellular level. The internalization of Gd-NB-TTTDA-IL11 was confirmed by in vitro MR imaging study (Figure 2). With the conjugates, the detection of the MDA-MB-231 cell line occurred with a noticeable MR contrast (T₁-weighted MR images). The signal intensity of positive cells in the present of Gd-NB-TTTDA-IL11 is significantly higher than those of positive cells in the absence of contrast agent. The enhancement of signal intensity was increased (57%) for the MDA-MB-231 cell line. Low signal intensity was observed for the negative cells in the presence of Gd-NB-TTTDA-IL11. The MTT assays using the MDA-MB-231 and HT1080 cell lines were performed to analyze the potential cytotoxicity of Gd-NB-TTTDA-IL11. In addition, Gd-NB-TTTDA-IL11 was shown lower cytotoxicity a (Figure 3.)

Conclusion

We have successfully prepared and characterized the Gd-NB-TTTDA-IL11 and Gd-NB-TTTDA-IL11-cy5.5. The dual-labeled IL11 peptide substrate probe can be used to detect IL-11Rα of cancer cells with fluorescence microscope at the cellular level. The results of in vitro optical and MR imaging indicated that it had high affinity for IL-11Rα, lower cell cytotoxicity and exhibited well imaging characteristics.

Figure 1. Fluorescence microscopy assays of Gd-NP-TTTDA-BN-Cy5.5 binding to IL-11Rα. The signals of Gd-NB-TTTDA-IL11-Cy5.5 (10μM) and DAPI are displayed in red and blue, respectively. (A) Binding to MDA-MB-231 cells. (B) Binding to HT-1080 cells.

Figure 2. The T₁-weighted images of MDA-MB-231 and HT-1080 cell after the treatment without or with [Gd (DTPA)]²⁻,[Gd (NP-TTTDA)]²⁻,Gd-NB-TTDA-IL11 and Gd-NB-TTTDA-IL11 with excess amount IL11 peptide. MRI was performed with a clinical 3.0-T MR scanner and animal coil, scanned by a Fast gradient echo pulse sequence (TR/TE = 150/5.8).

Figure 3. In vitro cell viability of as a function of Gd-NB-TTTDA-IL11 concentrations in MTT assay. Pink is MDA-MB-231 cell line. Purple is HT-1080 cell line.