In vivo molecular imaging using a dual-imaging reporter gene for verifying the role of HOXA gene subfamily in gastric cancer development and therapeutic monitoring

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Abstract

Molecular imaging methods have been used recently to investigate biologic events. To develop a molecular imaging method suitable for monitoring viable gastric cancer cells, we established a high penetration, high sensitivity infrared-fluorescent protein (IFP) for fluorescent imaging and synthesized the relatively high temporal and spatial resolution magnetic resonance(MR) sensing contrast agents (SCA): β-galactosidase (β-gal) MR probe for producing the MR molecular imaging with a dual-imaging reporter animal model. The modality would be useful for verifying the role of HOXA gene subfamily in gastric cancer development and therapeutic monitoring.

Introduction

Cancer so far is still one of the leading causes of human death, especially those which are difficult to be treated or easily to have metastasis. Most outstanding research institutes are making their efforts to understand the mechanism of carcinogenesis, metastatic pathway and tumor biomarkers for reaching the effective therapeutic methods. Our research group found that Hox A genes are over expressed in human gastric cancer tissues, particularly HoxA7, HoxA9 and HoxA13. Therefore, we hypothesized that non-invasive in vivo imaging may play an important role and research platform for investigating the pathophysiology in carcinogenesis of the gastric cancer and monitoring the effects of various therapies.

Magnetic resonance imaging (MRI) has high spatial resolution and soft-tissue contrast without irradiation hazard. However, the sensitivity of MRI is relatively lower at the molecular level. Synthesis of a tissue specific MRI contrast agent, β-galactosidase (β-gal) MRI probe can enhance the sensitivity of MR molecular imaging for detecting malignancies (1). In vivo optical imaging of deep tissues in animals is most feasible between 650 and 900 nm because such wavelengths minimize the absorbance by hemoglobin, water, and lipids, as well as light-scattering(2). Thus, genetically encoded IFP, with excitation and emission maxima of 684 and 708 nm, would be particularly valuable for whole-body imaging (3). In this study, utilized lentivirus as the vector, the transfection of IFP/β-gal gene fragments into human gastric cancer cells by means of biliverdin excited IFP fluorescent imaging and tissue specific MR contrast agent, β-gal probe, generated MR imaging. Thereby, we established two in vivo high sensitivity and specificity imaging modalities to verify the role of HOXA gene subfamily in gastric cancer development and therapeutic monitoring.

Methods

1. Recombinant DNA pLKO-AS3w-IFP and pLKO-AS2-β-gal (IFPs/β-gal) plasmid constructs.
2. Production of lentivirus with transgene IFP or β-gal
3. Gastric cancer cells transfection from MKN45 to MKN45/IFP-β-gal stable cloning cells, then the cells selection by puromycin and hygromycin, and then sorting by Flow Cytometry
4. Animal models: SCID mice bearing established MKN45 and MKN45/IFP-β-gal tumors by subcutaneously injected into their left & right thigh regions (5×10^6 cells).
5. Synthesized the a β-galactopyranose-containing gadolinium (III) complex [Gd(DOTA-FPG)(H_2O)] and characterized as being potentially suitable for a bioactivated MRI contrast agent (1).
6. In vivo imaging: The IFP channel was excited with a 650/50nm (center wavelength /full width at half maximum) band pass filter with a 700nm long pass filter in series with the imager's tunable emission filter at 710/40 nm by 250nmol intravenous injection of biliverdin. MR imaging was performed using a 3T MR scanner using a specially-designed animal receiver coil. T1-weighted FSE (TR/TE 833/11ms) axial images were obtained before and after intravenous injection of the contrast agent.
7. MR enhancement % = [SI tumor(post)/SD tumor(post) - SI tumor(pre)/SD tumor(pre)]/SI tumor(pre)/SD tumor (pre)x100, where SI tumor(post) and SI tumor(pre) are the MR mean signal intensities of the MKN45 tumor and MKN45/IFP- β -gal tumor before & after injection of 0.3mmol/kg [Gd(DOTA-FPG)(H_2O)]. The SD is the standard deviation.

Results and Discussion

The restriction enzyme cut by NheI、AscI showed that the pLKO-AS2-β-gal and pLKO-AS3w-IFP plasmids are correct (Figure 1) (pLKO-AS2: 8K; β-gal: 3K; pLKO-AS3w: 9K; IFP: 1K). The flow cytometry in Figure 2 showed normalized excitation and emission spectra of IFP and β-gal. In vivo fluorescence molecular images showed presence of fluorescence in the MKN45/IFP - β-gal tumor but no any fluorescence in MKN45 tumor (Figure 3). In vivo MR images of the animal and time-enhancement(%) change of the tumor were shown in Figure 4, indicating that [Gd(DOTA-FPG)(H_2O)] can act as a MRI contrast agent for noninvasive imaging of gene expression in vivo with the enhancement(%) was 44-67% in MKN45/IFP-β-gal tumor and 11-18% in MKN45 tumor .

Conclusion

The described modality using a dual-imaging reporter would be useful to verify the role of HOXA gene subfamily in gastric cancer development and might also play a role in evaluating the effects of various therapeutic approaches, such as chemotherapy, radiotherapy, gene therapy, and immunotherapy. Because of the advantages offered by a combination of optical and MR images, it is hopeful that this technique will move quickly from in vitro and animal study to clinical trials for the further personal or gene therapy monitoring of the gastric cancer.

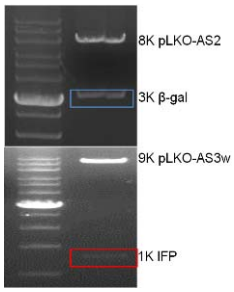

Figure 1. Restriction enzyme cut by NheI、AscI check IFPs and β-gal gene fragment

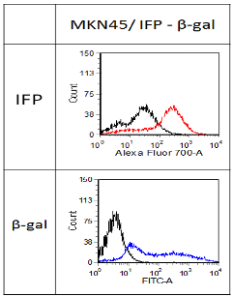

Figure 2. Flow cytometry of MKN45/IFP-β-gal stable cloning cells. Cloning cells without substrate stain (black), with biliverdin (red), or FDG(blue)

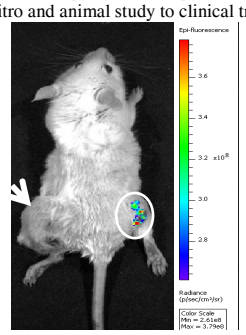

Figure 3. IVIS imaging after intravenous injection of biliverdin. MKN45 cells tumor(arrow) and MKN45/IFP - β-gal cells tumor (circle).

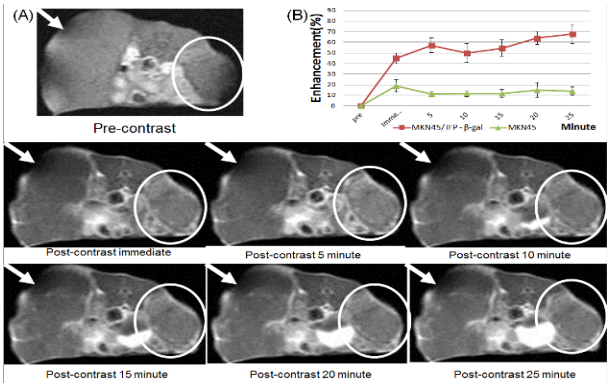

Figure 4. (A) In vivo MR images of SCID mouse before & after intravenous injection of [Gd(DOTA-FPG)(H_2O)] show MKN45 tumor(arrow) and MKN45/IFP- β -gal tumor (circle). (B)Time-enhancement change(%) of the MKN45 tumor and MKN45/IFP- β -gal tumor before & after injection of 0.3mmol/kg [Gd(DOTA-FPG)(H_2O)].

Acknowledgments

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