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Research Paper

Plectin-1 Targeted Dual-modality Nanoparticles for Pancreatic Cancer Imaging

Xiao Chen [a,b,1], Hao Zhou [a,1], Xiaoshuang Li [a], Na Duan [a], Shouyou Hu [c], Yongkang Liu [a], Yali Yue [a], Lina Song [a], Yifen Zhang [d], Donghui Li [e], Zhongqiu Wang [a,*]

[a] Department of Radiology, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing 210029, China
[b] Division of Nephrology, Zhongshan Hospital Fudan University, Shanghai 200032, China
[c] Department of Oncology, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing 210029, China
[d] Department of Pathology, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing 210029, China
[e] Department of Gastrointestinal Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

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ABSTRACT

Background: Biomarker-targeted molecular imaging holds promise for early detection of pancreatic cancer. The aim of this study was to design and evaluate a plectin-1 targeted multi-functional nanoparticle probe for pancreatic cancer imaging.
Methods: 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-amino(polyethylene glycol) (DSPE-PEG-NH2)-modified superparamagnetic iron oxide (Fe_3O_4) nanoparticles (SPION) were conjugated with plectin-1 antibody and/or Cy7 to create the multi-functional targeted nanoparticle targeted probe (Plectin-SPION-Cy7) or non-targeted probe (SPION-Cy7). Pancreatic carcinoma cell lines expressing plectin-1 were cultured with the targeted or control probes and then were imaged using confocal laser scanning microscopy and magnetic resonance imaging (MRI). Accumulations of the nanoparticles in pancreatic tumor xenografted mice were determined by MRI and fluorescence imaging.
Results: In vitro optical imaging and MRI showed that the targeted nanoparticles were highly accumulated in MIAPaCa2 and XPA-1 carcinoma cells but not in non-carcinoma MIN6 cells, which was further confirmed by Prussian blue staining. *In vivo* MRI showed a significant T2 signal reduction. Prussian blue staining further confirmed that the plectin-1 targeted nanoparticles were highly accumulated in the tumor mass but not in normal pancreatic tissues, or in the liver and kidney, and few nanoparticles were observed in the tumors of mice injected with SPION-Cy7.
Conclusions: Our data demonstrate that plectin-1 targeted fluorescence and MR dual-functional nanoparticle can visualize pancreatic cancer, and it has great potential to be used with various imaging devices for pancreatic cancer detection.

1. Introduction

Pancreatic cancer is a highly aggressive and rapidly fatal malignancy with a 5-year survival rate of <5% (Jemal et al., 2008). Surgical resection of the tumor is the only possible cure but most patients are usually diagnosed with unresectable late stage disease (Willett et al., 2005). Therefore, the prognosis is poor. Early detection at the potentially curable stage is critical to reduce the mortality of pancreatic cancer. However, conventional imaging technology, such as ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI) have not significantly increased our ability to detect early-stage disease or affect outcomes of pancreatic cancer patients (Cote et al., 2013). In addition, conventional imaging is limited in differentiating malignancy from benign lesions. Thus, overtreatment of patients with pancreatic benign cystic lesions could occur. Therefore, there is an unmet clinical need to develop agents for early detection. Endoscopic imaging and molecular-based radiographic tests hold the promise to help precisely identify pancreatic malignant lesions and their precursors at early stages. Furthermore, molecular imaging could improve our understanding of disease pathogenesis and identify diagnostic markers and therapeutic targets.

Recently, targeted molecular imaging has shown an advantage in early detection of cancer with high specificity and sensitivity in animal models (Weissleder, 2006). Optical imaging (fluorescence and bioluminescence), MR imaging, ultrasound, and positron emission tomography have been developed for *in vivo* molecular imaging (Massoud and Gambhir, 2003). Fluorescence imaging is usually used for its low costs of detection devices, easy use and interpretation, and absence of ionizing radiation (Ntziachristos, 2010). Near-infrared (NIR) imaging has the advantage of improved tissue penetration (up to 1 cm) and low

* Correspondence author.
E-mail address: zhq2001us@163.com (Z. Wang).
[1] Xiao Chen and Hao Zhou contributed equally to this work.

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tissue auto-fluorescence (Weissleder and Ntziachristos, 2003; Weissleder, 2006; Houghton et al., 2015). Magnetic nanoparticles (MNPs) can induce changes in T2-or T2*-weight MR images and have been considered as good carriers for targeted molecules with high biocompatibility and low toxicity (Vuong et al., 2012). Optical imaging and/or MR imaging has been applied for pancreatic cancer imaging in previous studies (Yang et al., 2009; Tong et al., 2013; Wang et al., 2016).

Targeted molecules are crucial in the design of molecular imaging probes. Targeted molecules should be highly expressed in cancer cells but absent or under-expressed in normal adjacent tissues (Yang et al., 2009). To find such target molecules, CA19.9 (Houghton et al., 2015), urokinase plasminogen activator (Yang et al., 2009), claudin-4 (Neesse et al., 2013), surviving(Tong et al., 2013; Wang et al., 2016), insulin-like growth factor-1 receptor (IGF-1R) (Park et al., 2016; England et al., 2016), integrin αvβ6 (Liu et al., 2014), integrin αvβ3 (Trajkovic-Arsic et al., 2014) and galectin-1 (Rosenberger et al., 2015) have been tested in pancreatic cancer imaging. Kelly et al. (2008) first identified that plectin-1 is highly expressed in invasive pancreatic cancer. Bausch et al. (2011) further showed that 93% of pancreatic ductal adenocarcinoma cases are plectin-1 positive, and the specificity and sensitivity of plectin-1 in distinguishing malignant from benign lesions are 83% and 84%, respectively. Konkalmatt et al. (2013) further showed that plectin-1 peptide targeted adeno-associated virus (AAV) vector could be selectively delivered to pancreatic carcinoma cells *in vivo*, which suggests applicability for early detection and treatment of pancreatic cancer. Wang et al. (2014) also showed that plectin-1 targeted dye bovine serum albumin (BSA)·superparamagnetic iron oxide nanoparticles (SPION)- monoclonal antibody (mAb) bioconjugates, but not the non-targeted bioconjugates could bind to the Panc-1 cells. Findings of these studies suggest that plectin-1 has the potential to be a molecular target for pancreatic cancer imaging. However, Konkalmatt et al. (2013) used AAV targeting peptide as the target molecule, and they only showed the optical imaging. Kelly et al. (2008) also used plectin-1 targeting peptide and only showed the MRI data of agar-embedded specimen. Wang et al. (2014) used the BSA-modified nanoparticles and only showed the efficiency of the probe in *in vitro* study. Further studies are required due to the limited number of imaging investigations using plectin-1 as a target.

In the present study, we designed a fluorescence and MR dual-modality magnetic nanoprobe targeting plectin-1 and evaluated its ability for pancreatic cancer imaging in both *in vitro* and *in vivo* experiments.

2. Materials and Methods

2.1. Detection of Plectin-1 Expression in Cell Lines

Plectin-1 protein expressions in four pancreatic cancer cell lines, MIAPaCa2, Panc-1, BxPC-3, XPA-1 and one pancreatic beta cell line MIN6 were determined using Western blot. Briefly, cellular protein was obtained using radioimmunoprecipitation assay (RIPA) lysis buffer. Thirty microgram of proteins was separated on a 7% Bis-Tris NuPAGE gel (Invitrogen) for 3 to 4 h at 60 v and blotted onto polyvinylidene difluoride (PVDF) membranes. The PVDF membrane was blocked with 5% (w/v) skim milk for 1 h at room temperature and then incubated with an anti-plectin-1 primary monoclonal antibody diluted as recommended by the manufacturer (Abcam, Cat# ab32528, MA, USA) at 4 °C overnight. The membrane was washed in TBST for three times, and then incubated with a secondary antibody conjugated with horseradish peroxidase at room temperature for 1 h. After washing, the bands were visualized using enhanced chemiluminescence. β-Actin was used as a loading control. Two independent experiments were performed.

2.2. Design and Synthesis of Plectin-SPION-Cy7 (Cyanine 7)

1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-amino(polyethylene glycol) was dispersed in chloroform and mixed with the superparamagnetic iron oxide (Fe_3O_4) nanoparticles (obtained as a general gift from Southeast University, Nanjing China) and deionized water. The mixture was then rotated and evaporated at 65 °C for 13 min to obtain NH2-PEG-SPIONs (NP-SPION). The oil-dispersible iron oxide nanoparticles can be modified with amphiphilic polymers by the hydrophobic interaction. The hydrophobic ligand of SPIONs binds with the hydrophobic end of amphiphilic polymers by the hydrophobic interaction. The hydrophilic group at the outer surface made the NP-SPION soluble. The solution was centrifuged (3000 rpm/min) for 5 min and dispersed in deionized water by using ultrasonic oscillation. The mixture was filtrated with a 0.22 μm filter several times before storage. The synthesized NP-SPION was mixed with 2-(N-Morpholino) ethanesulfonic acid buffer, and then carbodiimide hydrochloride (EDC) and sulfo- N-hydroxysulfosuccinimide (NHS) solutions. The mixture was shaken in a swing bed (150 rpm) for 30 min, centrifuged at 3000 rpm/min for 3 min, and then washed twice with borate buffer. Then, 20 μg of anti-plectin-1 monoclonal antibody (0.5 mg/ml, Abcam, MA, USA) and/or 700 μg of Cy7 NHS (Near infrared fluorescent, NIRF; Sigma-Aldrich, MO, USA) were added to get Plectin-SPION-Cy7 or SPION-Cy7. The Plectin-SPION-Cy7 was purified by gel chromatography. For SPION-Cy7, the unbound Cy7 was removed by centrifugation. The final solution was shaken in a swing bed for 12 h, and then stored away from light at 4 °C.

2.3. Characterizations of the Targeted Probe

The size of the targeted magnetic probe was determined using a transmission electron microscope (Joel, JEM-200CX, Japan). Briefly, the samples were dispersed in alcohol (25 μg Fe/ml), then deposited onto the double copper grids, carbon coated (Electron Microscopy Sciences, PA, USA) and left until the excess liquid volatilized, and then were dried under infrared light. Samples were imaged at an acceleration voltage of 180 kV. Then, the samples were diluted with deionized water. The hydrodynamic sizes and zeta potential of SPION, SPION-Cy7, and Plectin-SPION-Cy7 were measured using a Zetasizer (Beckman, Delsa 440SX, Germany) at pH of 7.0–7.2. The relaxivity values of r1 and r2 were calculated by fitting the 1/T1 and 1/T2 relaxation time (s^{-1}) *versus* Fe^{3+} concentration (mM) curves by using an NMR Analyzer(Bruker Optik GmbH, Ettlingen, Germany). The T2-weighted images were acquired using a T2-weighted spin echo pulse sequence with TR = 1200 ms, TE = 110 ms, field of view (FOV) = 100 × 120 mm^2, data matrix = 280 × 216, slice thickness = 2 mm by using 1.5 T MRI (AChieva 1.5 T, Philips, the Netherland). The precipitated solid was dried at 40 °C after washing three times. Magnetic properties were analyzed using vibrating sample magnetometry (VSM, Lakeshore 7407, USA) with a saturating field of 1.0 Tesla. The stability of SPION in sodium chloride (0 to 1.0 mM) solution (contain 5% BSA) and in the solutions of varying pH values (3 to 11) were also observed for 7 days. In addition, we analyzed the particle size and polydispersity index (PDI) *via* dynamic light scattering (DLS) using a Zetasizer at 1st and 7th days in phosphate-buffered saline (PBS) and 20% serum solution. The results are expressed as mean ± standard deviation ($n = 3$). Two independent characterizations were performed.

2.4. In Vitro Optical and MRI Imaging and Prussian Blue Staining

The MIAPaCa2, XPA-1 and MIN6 cells (1.0×10^5 cells/well) were plated in 6-well plates and cultured in Iscove's Modified Dulbecco's Medium (IMDM) or Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS; Sigma, USA), 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 with medium change every 2 days. Upon reaching 50–60% confluences, SPION-Cy7 and Plectin-SPION-Cy7 were added, and the cells were cultured for 24 h. Control cells did not receive any nanoparticles. The harvested cells were washed 3 times with PBS and fixed in 4% polyoxymethylene, stained using Hoechst Kit according to the

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