



Noninvasive small-animal imaging of galectin-1 upregulation for predicting tumor resistance to radiotherapy

Jianhao Lai^a, Dehua Lu^a, Chenran Zhang^a, Hua Zhu^b, Liquan Gao^a, Yanpu Wang^a, Rui Bao^a, Yang Zhao^a, Bing Jia^a, Fan Wang^{a,c}, Zhi Yang^{b,**}, Zhaofei Liu^{a,*}

^a Medical Isotopes Research Center and Department of Radiation Medicine, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China

^b Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Nuclear Medicine, Peking University Cancer Hospital & Institute, Beijing 100142, China

^c Key Laboratory of Protein and Peptide Pharmaceuticals, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

ARTICLE INFO

Article history:

Received 3 October 2017
Received in revised form
13 December 2017
Accepted 14 December 2017
Available online 15 December 2017

Keywords:

⁶⁴Cu
ImmunoPET
Galectin-1
Radiotherapy
Hypoxia

ABSTRACT

Increasing evidence indicates that the overexpression of galectin-1, a member of the galectin family, is related to tumor progression and invasion, as well as tumor resistance to therapies (e.g., radiotherapy). Herein, we investigated whether near-infrared fluorescence (NIRF) imaging and positron-emission tomography (PET) were sensitive approaches for detecting and quantitating galectin-1 upregulation *in vivo*. An anti-galectin-1 antibody was labeled with either an NIRF dye or ⁶⁴Cu, and NIRF and PET imaging using the resulting probes (Dye- α Gal-1 and ⁶⁴Cu-1,4,7-triazacyclononane-1,4,7-triacetic acid [NOTA]- α Gal-1) were performed in 4T1 breast cancer-bearing mice treated with several rounds of sorafenib. Radiotherapy was performed *in vitro* and *in vivo* to identify the role of galectin-1 in radioresistance. NIRF and PET imaging both revealed significantly increased upregulation of galectin-1 in the hypoxic tumors after sorafenib treatment, which was verified by *ex vivo* biodistribution, western blotting, and enzyme-linked immunosorbent assays. Galectin-1 specific inhibition by thiodigalactoside dramatically improved the efficacy of radiotherapy, and overcame sorafenib-induced radiotherapy resistance. Taken together, galectin-1 is a key mediator of tumor resistance to radiotherapy. Targeted molecular imaging allows for real-time, noninvasive, and quantitative detection of the dynamic changes in galectin-1 levels *in vivo*; this introduces the possibility of early detection of tumor resistance to therapies.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Galectins are members of huge carbohydrate-binding lectins, and are characterized by high-affinity binding to β -galactosides through a highly conserved carbohydrate recognition domain [1]. Among the 15 galectin family members identified to date in mammals [2], galectin-1 is the 14.5 kDa prototypal member of the galectin superfamily, and is expressed in a variety of tumors including those of the breast, lung, ovary, and colon as well as in gliomas, melanomas, and tumor endothelial cells [3]. Galectin-1 is involved in various cancer-related processes, such as tumor cell

aggregation, angiogenesis, cell migration and adhesion, and immunosuppression [4]. In most cancers, increased galectin-1 expression correlates with tumor aggressiveness and metastasis, and is associated with poor prognosis [5].

Resistance to radiotherapy is a pervasive problem in clinical oncology; one of the chief causes of radiotherapy resistance is tumor hypoxia [6]. Galectin-1 is linked to tumor hypoxia and radiotherapy resistance through its modulation of DNA damage repair, angiogenic rescue programs, and cytotoxic T cell apoptosis [7,8]. Galectin-1 is highly expressed in prostate cancer [9] and melanoma [10], which are well known to be radioresistant; meanwhile, the expression of galectin-1 is not detectable in radiosensitive Hodgkin's lymphoma [11]. Considering its various pro-tumor functions and abundant tumor expression profile, a molecular imaging strategy that can noninvasively monitor tumor galectin-1 expression might provide useful information about tumor aggressiveness,

* Corresponding author.

** Corresponding author.

E-mail addresses: pekyz@163.com (Z. Yang), liuzf@bjmu.edu.cn (Z. Liu).

patient prognosis, and resistance to radiotherapy. Molecular imaging of galectin-1 expression could also constitute a robust tool for noninvasive detection of the changes associated with cancer interventions that are related to hypoxia *in vivo*.

Sorafenib, a small-molecule multikinase inhibitor, is approved for the treatment of metastatic renal cell carcinoma and advanced hepatocellular carcinoma [12]. The antitumor effects of sorafenib in these two diseases are, at least in part, attributed to the antiangiogenic effects of this agent through its inhibition of growth factor receptors [13]. However, the transient response and the greater invasiveness of relapsed tumors limit sorafenib's benefit on overall survival [14]; this may be explained by increased tumor hypoxia [15] and the recruitment of M2-type tumor-associated macrophages [16,17] after sorafenib therapy. The administration of antiangiogenic agents such as sorafenib has been shown to generate intratumoral hypoxia, which can modulate tumor progression [18]. It has also been reported that hypoxia-induced galectin-1 upregulation may serve as a biomarker for sorafenib resistance, while galectin-1 knockdown was shown to restore sensitivity [19].

In this study, we developed near-infrared fluorescence (NIRF) and positron-emission tomography (PET) imaging probes to non-invasively visualize and quantify galectin-1 levels in tumors that undergo hypoxia induced by sorafenib treatment *in vivo*. We investigated whether molecular imaging of galectin-1 expression could provide information regarding sorafenib-induced tumor hypoxia and resistance to radiotherapy.

2. Materials and methods

2.1. Cell culture and animal model

The 4T1 murine breast cancer, CT26 murine colon carcinoma, and B16-F10 murine melanoma cell lines were purchased from American Type Culture Collection. Human embryonic kidney HEK293 cells were kindly provided by Professor Tao Xu (Institute of Biophysics, China Academy of Science). 4T1 and CT26 cells were grown in RPMI-1640 medium, and B16-F10 and HEK293 cells were grown in high-glucose Dulbecco modified Eagle medium. All cell lines were cultured in medium supplemented with 10% fetal bovine serum at 37 °C in humidified atmosphere containing 5% CO₂.

All animal experiments were performed by following the protocol approved by the Institutional Animal Care and Use Committee at Peking University. The tumor-bearing mouse model was established by subcutaneous injection of 1×10^6 4T1 cells into the female BALB/c mice (5 weeks of age; Department of Laboratory Animal Science, Peking University). Tumor growth was measured using a caliper, and the tumor volume was calculated using the formula: volume = length \times width²/2.

2.2. Western blotting

Expression of galectin-1 in different cell lines (4T1, CT26, B16-F10, and HEK293) and in 4T1 tumor cells and tissues following different treatments was detected by western blotting. For the cellular hypoxia mimetic assay, 4T1 cells were treated with different doses (0, 100, and 200 μ M in cell culture medium) of cobalt chloride (CoCl₂) for 24 h, and galectin-1 levels were detected by western blotting. Briefly, tumor tissues or cells were homogenized and proteins were extracted using RIPA buffer (CWBio, Beijing, China). Protein concentrations were determined using a microBCA protein assay kit (CWBio, Beijing, China). After sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transfer to polyvinylidene difluoride (PVDF) membranes, these membranes were blocked using 5% nonfat milk blocking buffer, and incubated

overnight at 4 °C with anti-galectin-1 primary antibody (1:1000; R&D Systems, Minneapolis, MN) or anti-hypoxia-inducible factor (HIF)-1 α primary antibody (1:200; Cayman, Ann Arbor, MI). Membranes were then incubated at 37 °C for 1 h with DyLight549-conjugated secondary antibodies (EarthOx, Millbrae, CA). β -actin was used as the loading control, and bands were detected using the Molecular Imager PharosFX™ Plus System (Bio-Rad, Hercules, CA). Western blot band intensities were quantitated using the ImageJ software (NIH, Bethesda, MD).

2.3. Synthesis and *in vitro* characterization of galectin-1-targeted imaging probes

The galectin-1-targeted NIRF and PET imaging probes were prepared by labeling a rat anti-mouse galectin-1 antibody (α Gal-1; R&D Systems, Minneapolis, MN) with DyLight755 and ⁶⁴Cu, respectively. DyLight755-conjugated α Gal-1 (Dye- α Gal-1) was prepared using a previously described method [20]. Briefly, α Gal-1 was mixed with DyLight755-NHS ester (Pierce, Rockford, IL) in sodium bicarbonate buffer (pH 8.5) at a 1:10 M ratio. After a 12 h incubation at 4 °C, Dye- α Gal-1 was purified using a PD-10 desalting column (GE Healthcare, Piscataway, NJ). An isotype-matched control probe (Dye-IgG) was synthesized by conjugating rat IgG with DyLight755-NHS ester using the same protocol.

For synthesizing ⁶⁴Cu-labeled α Gal-1, α Gal-1 was first conjugated with *p*-SCN-Bn-NOTA (Macrocyclics, Dallas, TX) in sodium bicarbonate buffer (pH 8.5) at a 1:200 M ratio. After a 12 h incubation at 4 °C, NOTA-conjugated antibody (NOTA- α Gal-1) was purified using a PD-10 desalting column. ⁶⁴CuCl₂ was produced in a Sumitomo HM-20 biomedical cyclotron via a ⁶⁴Ni(p,n)⁶⁴Cu reaction [21]. For radiolabeling, 80 μ g of NOTA- α Gal-1 was reacted with 222 MBq of ⁶⁴CuCl₂ in 300 μ L of 0.1 M sodium acetate buffer (pH 5.5) at 37 °C for 40 min with constant shaking. The final product, ⁶⁴Cu-NOTA- α Gal-1, was purified on a PD-10 columns using phosphate-buffered saline (PBS) as the mobile phase. ⁶⁴Cu-labeled isotype-matched control probe (⁶⁴Cu-NOTA-IgG) was synthesized using the same protocol.

The *in vitro* galectin-1 binding affinity, and the specificity of the Dye- α Gal-1 and NOTA- α Gal-1, were assessed via a competition binding assay using ¹²⁵I- α Gal-1 as the galectin-1 radioligand. ¹²⁵I- α Gal-1 was prepared by labeling α Gal-1 with Na¹²⁵I using the Iodogen method as previously described [17]. ¹²⁵I- α Gal-1 (7.4 kBq) was added to 96-well Stripwell™ enzyme-linked immunosorbent assay (ELISA) plates (Costar, Cambridge, MA) coated with 0.2 μ g galectin-1 protein (R&D Systems, Minneapolis, MN) in the presence of increasing concentrations of α Gal-1, Dye- α Gal-1, or NOTA- α Gal-1 (0–333 nM). After incubation for 1 h at 37 °C, the plates were washed with PBS, and wells were collected and measured in a gamma counter (Packard, Meriden, CT). The best-fit 50% inhibitory concentration (IC₅₀) values were calculated via nonlinear regression plots using Prism 5.0 (GraphPad Software, San Diego, CA). Each experiment was performed twice using quadruple samples.

The galectin-1 binding specificity of ⁶⁴Cu-NOTA- α Gal-1 was also evaluated in the 96-well Stripwell™ ELISA plates coated with 0.2 μ g galectin-1 protein. Briefly, 7.4 kBq ⁶⁴Cu-NOTA- α Gal-1 was added to the plates with or without an excess amount of α Gal-1 (20 μ g/mL) and then incubated at 37 °C for 1 h, after which the plates were washed with PBS and wells were collected and measured in a gamma counter.

2.4. *In vivo* NIRF imaging

For *in vivo* galectin-1-targeted NIRF imaging, each 4T1 tumor-bearing mouse ($n = 5$ per group) was administered 0.5 nmol of Dye- α Gal-1 or control Dye-IgG intravenously. At 4, 8, 24, 48, 72, and

متن کامل مقاله

دریافت فوری ←

ISIArticles

مرجع مقالات تخصصی ایران

- ✓ امکان دانلود نسخه تمام متن مقالات انگلیسی
- ✓ امکان دانلود نسخه ترجمه شده مقالات
- ✓ پذیرش سفارش ترجمه تخصصی
- ✓ امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
- ✓ امکان دانلود رایگان ۲ صفحه اول هر مقاله
- ✓ امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
- ✓ دانلود فوری مقاله پس از پرداخت آنلاین
- ✓ پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات