Cancer Letters 356 (2015) 891–898

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Original Articles

In vivo molecular imaging of gastric cancer in human-murine xenograft models with confocal laser endomicroscopy using a tumor vascular homing peptide

Lijuan Liu [a,1], Jipeng Yin [a,1], Changhao Liu [a,1], Guofeng Guan [b], Doufei Shi [c], Xiaojuan Wang [a], Bing Xu [a], Zuhong Tian [a], Jing Zhao [d], Yongzhan Nie [a], Biaoluo Wang [a], Shuhui Liang [a,*], Kaichun Wu [a,**], Jie Ding [a,***]

[a] State Key Laboratory of Cancer Biology and Xijing Hospital of Digestive Diseases, Xijing Hospital, Fourth Military Medical University, Xi'an, China
[b] Orthopaedic Oncology Institute, Tangdu Hospital, Fourth Military Medical University, Xi'an, China
[c] Department of Geriatrics, Affiliated Hospital of Binzhou Medical University, Binzhou, China
[d] Department of Obstetrics and Gynecology, Xijing Hospital, Fourth Military Medical University, Xi'an, China

ARTICLE INFO

Article history:
Received 14 August 2014
Received in revised form 29 October 2014
Accepted 30 October 2014

Keywords:
Gastric cancer
GEBP11
Confocal laser endomicroscopy
Molecular imaging

ABSTRACT

The early detection of premalignant lesions and cancers are very important for improving the survival of patients with gastric malignancies. Confocal laser endomicroscopy (CLE) is a novel imaging tool for achieving real-time microscopy during the ongoing endoscopy at subcellular resolution. In the present study, to evaluate the feasibility of real-time molecular imaging of GEBP11 by CLE in gastric cancer, CLE was performed on two types of tumor-bearing mice models, as well as surgical specimens of patients with gastric cancer, after the application of GEBP11. A whole-body fluorescent imaging device was first used to screen for the strongest specific fluorescent signal in xenograft models. Next, the tumor sites, as well as human tissues, were scanned with CLE. After this, targeted specimens were obtained for fluorescence microscopy and histology. We confirmed that GEBP11 could specifically bind to co-HUVECs by means of CLE in cell experiments. Thereafter, a specific signal was observed in both subcutaneous and orthotopic xenograft models in vivo after the injection of FITC-GEBP11 via tail vein, whereas the group injected with FITC-URP showed no fluorescent signals. In human tissues, a specific signal of GEBP11 was observed in 26/28 neoplastic specimens and in 8/28 samples of non-neoplastic specimens from the patients ($p < 0.01$). The findings from ex vivo immunofluorescence microscopy of cryostat sections correlated well with that obtained by CLE. These findings indicate that the peptide, GEBP11, might be a potential candidate for the molecular imaging of gastric cancer.

Introduction

Gastric cancer is the second leading cause of cancer-related deaths worldwide, and almost 990,000 cases of gastric cancer are detected annually [1]. Early detection has been associated with significantly improved patient survival. Researchers have found that angiogenesis and vasculature play important roles in the growth and metastasis of solid tumors [2]. Additionally, it has been reported that tumor vessels have many different characteristics from normal vessels [3–8], especially regarding the expression of specific molecules, such as GEBP11 [9]. GEBP11 is a new nine amino acid vascular homing peptide, which was screened and identified using phage display technology. In our previous work, we confirmed the specific binding affinity of GEBP11 to the vasculature of gastric cancer tissue and its outstanding performance in tumor imaging [9,10]. These data showed that GEBP11 could serve as an important candidate for tumor molecular imaging.

Endoscopy is considered as the gold standard for diagnosing of gastric cancer and is widely used in the clinic [11–16]. However, its usefulness may be limited because endoscopy is usually used for the gross examination of the mucosal surface and a biopsy is needed to make the final histopathologic diagnosis, which is subject to embedding, cutting and staining artifacts [17,18]. In recent years, to increase the accuracy of diagnosis, some special, high

Abbreviations: CLE, confocal laser endomicroscopy; FITC, fluorescein isothiocyanate; HUVECs, human umbilical vein endothelial cells; co-HUVECs, co-culture human umbilical vein endothelial cells; FACS, fluorescence-activated cell sorting; H&E, hematoxylin and eosin.

* Corresponding author. Tel.: +86 29 84771587; fax: +86 29 82539041.
E-mail address: liangsh@fmmu.edu.cn (S. Liang).
** Corresponding author. Tel.: +86 29 84771502; fax: +86 29 82539041.
E-mail address: kaicwu@fmmu.edu.cn (K. Wu).
*** Corresponding author. Tel.: +86 29 84771504; fax: +86 29 82539041.
E-mail address: dingjie@fmmu.edu.cn (J. Ding).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.canlet.2014.10.036
0304-3835/

definition endoscopic techniques, including chromoendoscopy, narrow band imaging, magnifying endoscopy, have emerged [19–24]. However, they have several disadvantages, the most notable of which is their lack of specificity. Therefore, an effective and alternative diagnosis strategy is urgently needed.

Confocal laser endomicroscopy (CLE) is an emerging tool that allows for the non-invasive, in vivo and real-time visualization of the gastrointestinal mucosal tissue with 1000 × magnification during the ongoing endoscopy [25–28]. In most trials, fluorescein sodium or acriflavine has been used as the contrast agent, and images with high resolution have been achieved. However, due to the lack of specificity, this application is limited in its utility for the diagnosis of gastrointestinal diseases. Currently, fluorescently labeled antibodies or peptides are used in the field of molecular imaging by using CLE [29–32]. By observing the targeted molecules, we can scan a large area of tissues in vivo to distinguish suspicious lesions or lesions, avoiding excessive biopsy. Although many studies have recently been reported on molecular imaging with CLE, as far as we know, no trials have been performed using labeled peptides specially targeting vasculature of gastric cancer. Thus, we hypothesized that fluorescently labeled GEBP11 may be observable by CLE in rodent models of human gastric cancer. Therefore, the aim of this study was to explore CLE for the in vivo molecular imaging of fluorescein isothiocyanate (FITC)-conjugated GEBP11 in two tumor-bearing xenograft models and in human gastric cancer samples.

Materials and methods

Cell culture and characterization

FITC-GEBP11 was synthesized by GL Biochem (Shanghai) Ltd and was examined and analyzed by MALDI-TOF MS and high-performance liquid chromatography (HPLC) systems. The human gastric cancer cell line, SGC7901, was generated in our laboratory and was cultured in Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Carlsbad, CA, USA) [33]. Human umbilical vein endothelial cells (HUVECs) were prepared as previously described and cultured in M200 basal culture media supplemented with low serum growth supplement (LSGS; Cascade Biologics, Portland, OR, USA) [9,34]. The tumor-endothelial cell co-culture model was established, and the co-culture of human umbilical vein endothelial cells (co-HUVECs) were prepared in vitro, as described previously [9,34,35]. All of these cells were grown in medium supplemented with 10% heat-inactivated fetal calf serum (Gibco, Grand Island, NY, USA), penicillin (100 U/mL) and streptomycin (100 mg/mL). The cells were maintained at 37 °C in an incubation chamber with 5% CO_2. Co-HUVECs, HUVECs and SGC7901 cells were harvested after 24–48 h during the log-growth phase and characterized by fluorescence-activated cell sorting (FACS) analysis, CLE and bench top fluorescent microscopy. For FACS, 10^5 tumor cells were incubated with 2 μL of FITC-conjugated GEBP11, unrelated peptide (URP), or phosphate-buffered saline (PBS). Additionally, 10^5 cells were cultured on chamber slides (Thermo, Waltham, MA, USA) and incubated with the FITC-GEBP11 without fixation. CLE (Cellvizio, Mauna Kea Technologies, Paris, France) was performed on these cells using exactly the same settings as used for the in vivo imaging of mice. Then, after CLE, bench top confocal laser microscopy (Olympus, Tokyo, Japan) of the cells was performed after nuclear counterstaining with 4', 6-Diamidine-2'-phenylindole dihydrochloride (DAPI, Roche, Basel, Switzerland) [29–31,36].

Mouse models

In this animal study, two different mouse models bearing gastric carcinoma were examined. Human gastric carcinoma xenografts were induced in 4–6 week-old athymic nude mice weighing 15–20 g. For the subcutaneous models, 5×10^6 gastric carcinoma SGC7901-Luc cells were injected into the right upper limb per mouse. In the orthotopic models, 5×10^6 cells were injected into the sub-serosa layer of the stomach wall after a small left lateral incision was made, as described previously [33]. Tumor progression was tracked dynamically by bioluminescence imaging (BLI). When the tumor size was 5–10 mm, the xenograft was exposed for imaging. The procedures were approved by the Animal Welfare and Ethics Committee of Fourth Military Medical University (FMMU).

Whole body and ex vivo organs fluorescent imaging

To visualize the fluorescence signal of FITC-GEBP11 in the whole animal in these two models, the small animal imaging system (IVIS Kinetics, Caliper Life Sciences, Hopkinton, MA, USA) was used. Mice (six for each model) were anesthetized with isofluorane and subsequently received FITC-GEBP11 or FITC-URP (1 μg/g body weight) via tail vein injection for both models. Fifteen minutes after the injection of the peptides, the small animal imaging system was used to obtain the peak intensity signal once every half hour [33]. After the whole-body fluorescent imaging of the tumor-bearing mice, the mice were sacrificed by 10% chloral hydrate (3 μL/kg i.p.) overdose via injection, and various organs (tumor, heart, liver, spleen, lung, kidney, stomach, intestine, bone, muscle, brain) were excised. The organs were then imaged to analyze the signal distribution of FITC-GEBP11 and FITC-URP and to determine where the signal intensity was the highest. The bioluminescent intensities of the regions of interest (ROIs) were measured by Living Image version 4.2 Software. Fluorescent intensity was presented as (photons/sec/cm^2/sr)/(μW/cm^2).

In vivo confocal laser endomicroscopy imaging

In vivo CLE in two murine models of human gastric cancer were performed according to an imaging protocol (Fig. 1). CLE imaging was carried out using a dedicated endomicroscopy system, with an excitation wavelength of 488 nm and light emission of 505–585 nm. For in vivo CLE imaging, FITC-GEBP11 (1 μg/g body weight) was

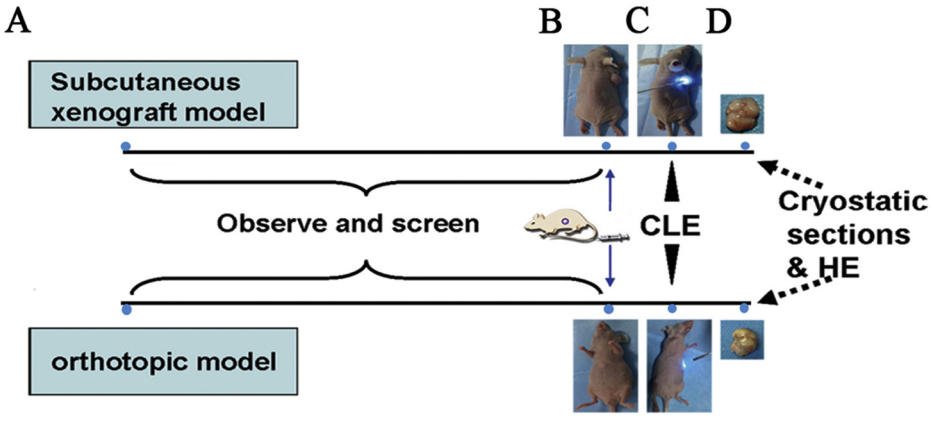

Fig. 1. In vivo molecular confocal laser endoscopy (CLE) in two murine models of human gastric cancer: imaging protocol. (A) Subcutaneous xenograft and orthotropic models were built. After a series of observation and screening studies, (B) FITC-GEBP11 or FITC-URP was injected intravenously in both models when the tumor was 5–10 mm (blue arrows). (C) After being shielded from light for 24 h after injection, CLE was performed (filled arrowheads). After CLE imaging, the tumors were collected for (D) cryosectioning and H&E (broken symbols) analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)