Contrast-enhanced magnetic resonance imaging with a novel nano-size contrast agent for the clinical diagnosis of patients with lung cancer

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Abstract. Recent studies have indicated that magnetic resonance imaging (MRI) efficiently diagnoses lung cancer. However, the efficacy of MRI in diagnosing lung cancer requires improving for patients in the early stage of the disease. In the present study, a novel nano-sized contrast agent of chistosan/Fe_3O_4-enclosed bispecific antibodies (BsAbCENS) was introduced, which targeted carcino-embryonic antigen (CEA) and neuron-specific enolase (NSE) in lung cancer cells. The diagnostic efficacy of contrast-enhanced MRI with BsAbCENS (CEMRI-BsAbCENS) was investigated in a total of 182 patients with suspected lung cancer who had high serum levels of CEA and NSE. BsAbCENS was administered by pulmonary inhalation prior to the MRI scan. The results revealed that CEA and NSE were overexpressed in human lung cancer cell lines. BsAbCENS bound with CEA and NSE on the surface of human lung cancer cells and produced a higher signal intensity than MRI alone for the diagnosis of patients with lung cancer. The diagnostic data revealed that CEMRI-BsAbCENS diagnosed 124/182 lung cancer cases, whereas CEMRI only diagnosed 98/182, which was significantly less ($P<0.01$). In addition, the survival rate of patients with lung cancer diagnosed by CEMRI-BsAbCENS was significantly higher than the mean 5-year survival rate ($P<0.01$). Furthermore, the pharmacodynamics demonstrated that BsAbCENS was metabolized within 24 h. The results of the present study indicate that the efficacy and accuracy of lung cancer diagnosis are improved by CEMRI-BsAbCENS. In conclusion, these results provide a potential novel protocol for the diagnosis of tumors in patients with suspected early stage lung cancer.

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Introduction

Lung cancer is one of the most prevalent types of human cancer and is the second highest cause of cancer-associated mortality worldwide (1). Previous studies have indicated that lung cancer is typically diagnosed when the disease has reached an advanced stage (2,3). There are different types of lung cancer, including large cell carcinoma, squamous cell carcinoma and adenocarcinoma, and the incidence rates of all types are increasing (4,5). The ability of physicians to diagnose lung cancer using imaging techniques has a substantial impact on the therapeutic decisions that are made and the survival time of patients (6). A missed diagnosis of lung cancer based on imaging tests may deny patients the opportunity to receive effective cancer therapy and result in a diminished survival time (7-9). Therefore, more sensitive methods of cancer diagnosis are required to improve therapeutic options and increase the 5-year survival rate (10).

At present, ultrasound, fludeoxyglucose-positron emission tomography (PET), computed tomography (CT) and magnetic resonance imaging (MRI) are widely used to diagnose and determine the disease stage of human lung cancer (11,12). In a clinical setting, MRI represents the most sensitive and accurate method of diagnosis for lung cancer and metastases (13). A previous study has indicated that the efficacy of contrast-enhanced (CE)MRI in diagnosing brain metastasis in lung cancer is increased compared with regular MRI scanning (14). Additionally, a recent comparison between PET/CT and MRI for diagnosis, staging and follow-up of patients with lung cancer revealed that MRI is useful for distinguishing benign and malignant pulmonary nodules,

has a higher sensitivity and specificity for nodal staging and is more beneficial in evaluating an early response to systemic chemotherapy (9). Freedman *et al* (15) have previously demonstrated that nanodelivery of MRI contrast agent (scL-gad-d nanocomplex) enhances the sensitivity of MRI to detect lung cancer metastases. Liposomal-iodinated contrast agent may facilitate the early detection and diagnosis of pulmonary lesions, and have implications on treatment response and monitoring (16). Additionally, although a number of reports have introduced various diagnoses of early-stage lung cancer by CEMRI using a contrast agent, nano-particle sized contrast agents present additional benefits than other contrast agents for the diagnosis of lung cancer, including high sensitivity and specificity (17-19). Therefore, in the present study the auxiliary role of chistosan/Fe_3O_4-encapsulated bispecific antibodies (BsAbCENS) in CEMRI-diagnosed lung cancer was investigated.

At present, tumor markers carcinoembryonic antigen (CEA) and neuron-specific enolase (NSE) are sensitive methods used for the diagnosis and treatment of lung cancer (20). Additionally, the combined detection of hematoporphyrin, CEA, NSE and CYFRA21-1 have been reported to significantly improve the sensitivity and specificity of lung cancer diagnosis, and may be useful for pathological typing (21). In the present study, the efficacy of CEMRI-BsAbCENS in the diagnosis of patients with lung cancer was investigated. It was revealed that CEMRI-BsAbCENS improves the signal intensity at the lung cancer location and also enhances the accuracy and sensitivity of MRI in the diagnosis of clinical patients with suspected lung cancer.

Materials and methods

Targeted contrast agent. Nano-sized chistosan/Fe_3O_4-enclosed bispecific antibodies (BsAbCENS, obtained from the department of bio-pharmaceuticals, Shandong University, Jinan, China) were produced using the covalent bond method as previously described (22). The bsAbCENS was taken using an atomizer (NE-J01; Contec Medical Systems Co., Ltd., Qinhuangdao, China). It was possible to visualize the nano-particles contrast agent via an MRI system. The BsAbCENS contrast agent was administered via atomizer 30, 60 and 90 min prior to MRI.

Patients. The inclusion and exclusion criteria of the present study were the same as previously reported (23). A total of 182 patients (104 males and 78 females; mean age, 48.4 years) with suspected early stage lung cancer (NSE ≥15 μg/ml; CEA >10 μg/ml) were enrolled in the present study from Shandong Medical Imaging Research Institute (Jinan, China) between May 2011 and July 2016. Patients were diagnosed according to the European Society for Medical Oncology clinical practice guidelines for the diagnosis of lung cancer (24).

A total of 182 healthy volunteers (105 males, 77 females; mean age, 48.6 years) were recruited from Shandong Medical Imaging Research institute (Jinan, China). All patients and healthy volunteers underwent a CEMRI scan and CEMRI-BsAbCENS for the detection of early-stage lung cancer. The characteristics of all participants within the present study are summarized in Table I.

The experiments in the present study were performed in accordance with the recommendations of the Guide for the Care and Use of Clinical Study of China (approval no. BUCMT20070612M25). The present study was approved by ethics committee of Shandong Medical Imaging Research Institute (Jinan, China) and all patients provided written informed consent prior to their inclusion within the present study.

Cells and reagents. Lung cancer cell line A549 and normal lung cell line MRC-5 were purchased from the American Type Culture Collection (Manassas, VA, USA). A549 cells were cultured in Dulbecco's modified Eagle medium (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) supplemented with 10% fetal bovine serum (FBS; Invitrogen; Thermo Fisher Scientific, Inc.). MRC-5 cells were cultured in 1640 medium (Sigma-Aldrich; Merck KGaA) supplemented with 10% FBS. All cells were cultured at 37˚C in a 5% CO_2 humidified atmosphere.

ELISA. The serum concentration levels of CEA (cat. no. DY4128), fibroblast growth factor receptor (cat. no. 661FR) and NSE (cat. no. DY5169-05; all R&D Systems, Inc., Minneapolis, MN, USA) were analyzed by commercialized ELISA kits. The operational procedures were performed according to the manufacturer's protocol. The results were analyzed using an ELISA reader system (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

MRI scanning. An MRI diagnosis system was used to diagnose patients with suspected lung cancer using preprogrammed settings. The settings were optimized to provide the optimal image formation. The whole lungs of all patients in the present study underwent MRI scanning according to the manufacturer's protocol (Ingenia 1.5T CX; Philips Medical Systems, Inc., Bothell, WA, USA). The details of the principles and settings used for MRI scanning were described in a previous study (25).

Image analysis. The outcomes generated by MRI scanning were analyzed using integral software in the MRI machine. Lung cancer location was diagnosed by three physicians using an image produced by the MRI. Patients with lung cancer were analyzed using an MRI scan and the signal enhancement of MRI induced by BsAbCENS was also measured via a pre-prepared program (T1-weighted, T2-weighted and fluid-attenuated inversion recovery sequences) in the MRI machine.

Treatments of patients with lung cancer diagnosed by BsAbCENS-MRI. All Patients with suspected early-stage lung cancer diagnosed via CEMRI-BsAbCENS received different treatments, including chemoradiotherapy, Traditional Chinese Medicine, biological therapy and comprehensive therapy after surgery. The clinical treatment methods for patients with lung cancer are listed in Table II. The median overall survival rate was analyzed as previously described (26).

Immunofluorescence and histological staining. BsAbCENS (2 mg/ml) was labeled with fluorescein isothiocyanate (FITC; 1 mg/ml in dimethylsulphoxide) as described in a

Table I. Characteristics of study patients.

Characteristic	Patients	Healthy volunteers
Male (n)	104	105
Female (n)	78	87
Age range (years)	28.8-64.6	28.6-64.8
Medical history of cancer (n)	8	0
Serum CEA (μg/l)	29.4±13.3	2.6±1.8
Serum NSE (μg/ml)	27.8±15.0	7.8±4.1

CEA, carcino-embryonic antigen.

Table II. Treatment of patients with lung cancer.

Treatment	Male (n=78)	Female (n=52)
Chemoradiotherapy	40	27
Chinese medicine	10	13
Biological therapy	14	5
Comprehensive therapy following surgery	14	7

previous study (27). Prior to immunofluorescence staining, A549 and MRC-5 cells were cultured to 85% confluence. All cells were fixed with 10% paraformaldehyde for 30 min at 37˚C and subsequently incubated with rabbit anti-human FITC-labeled antibodies targeting CEA (1:1,000; cat. no. ab133633, Abcam, Cambridge, UK) or NSE (1:1,000; cat. no. ab53025; Abcam) for 1 h at 25˚C. The cells were washed three times with PBS and observed using a fluorescence microscope (CKX53; Olympus Corporation, Tokyo, Japan). For histological staining 4-μm-thick tumor sections were fixed with 10% formaldehyde for 10 min at 37˚C and then stained with hematoxylin and eosin for 1 h at 37˚C as previously described (28).

Statistical analysis. All data are presented as the mean ± standard deviation of 3 experiments. Unpaired data was analyzed using Student's t-test. Kaplan-Meier analysis was used to estimate the survival rate every 5-month call visits during 60 months observation (to observe overall survival rate and tumor recurrence). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics of patients. A total of 182 patients with suspected lung cancer and 182 age-matched healthy volunteers were enrolled in the present study. The age range was 28.8-64.6 and 28.6-64.8 years in the patients and healthy volunteers, respectively. The numbers of male and female participants were similar in the patient and healthy volunteer groups. The characteristics of all patients included in the present study are summarized in Table I.

Analysis of the serum expression of CEA and NSE in lung cancer tissues and normal cells. The serum levels of CEA and NSE were analyzed in patients with lung cancer and healthy volunteers. It was observed that the serum levels of CEA and NSE were significantly higher in patients with lung cancer compared with healthy individuals (Fig. 1A and B). It was demonstrated that CEA and NSE were overexpressed in lung cancer tissues compared with the adjacent normal tissues (Fig. 1C). Immunofluorescence analysis revealed that the expression levels of CEA and NSE were upregulated in the A549 lung cancer cell line compared with the MRC-5 normal lung cell line (Fig. 1D). It was also demonstrated that BsAbCENS notably decreased the CEA and NSE expression levels in lung cancer cells (Fig. 1E). These results indicate that patients with lung cancer exhibited higher plasma CEA and NSE levels than healthy volunteers.

Efficacy of CEMRI-BsAbCENS in early clinical diagnosis for patients with suspected lung cancer. The diagnostic efficacy of CEMRI-BsAbCENS was investigated in patients with suspected lung cancer. The dose of CEMRI-BsAbCENS that provided the optimal signal intensity for CEMIR detection was 24 mg/kg for 30 min (Fig. 2A). CEMRI-BsAbCENS diagnosed 122 (68.13%; 76 male and 46 female) patients with lung cancer, whereas CEMRI diagnosed 98 (53.85%; 62 male and 36 female) patients with lung cancer. This demonstrated that CEMRI-BsAbCENS diagnosed a significantly higher number of patients with lung cancer compared with CEMRI (Fig 2B). Following 60 min inhalation of BsAbCENS it was also observed that there were significantly decreased serum concentrations of CEA and NSE in patients with lung cancer compared with those who had not inhaled BsAbCENS (Fig. 2C and D). These results suggest that CEMRI-BsAbCENS is more effective than CEMRI due to binding with CEA and NSE for the diagnosis of lung cancer than CEMRI.

Histopathology confirms the diagnosis of CEMRI-BsAbCENS for lung cancer cases. Histopathology images were used to further confirm the results of the CEMRI-BsAbCENS and CEMRIs. It was demonstrated that the BsAbCENS contrast agent improved image quality generated by MRI (Fig. 3A). The representative images demonstrate that CEMRI-BsAbCENS defined the image of the tumor more clearly than the CEMRI (Fig. 3B). The CEMRI-BsAbCENS diagnosis of patients with lung cancer was confirmed by histological analysis of lung tissues (Fig. 3C). Histopathology confirmed that 130 (78 male patients and 52 female patients) patients had lung cancer and CEMRI-BsAbCENS diagnosed 124 of these patients. The diagnostic rate was significantly higher for CEMRI-BsAbCENS compared with CEMRI for patients with suspected lung cancer (Fig. 3D). These results suggest that CEMRI-BsAbCENS is accurate in diagnosing patients with lung cancer.

Pharmacodynamics of BsAbCENS in the serum of patients with lung cancer. The signal intensity of CEMRI-BsAbCENS following the administration of BsAbCENS was investigated in patients with lung cancer. The results revealed that BsAbCENS was almost fully metabolized from the blood at 24 h following

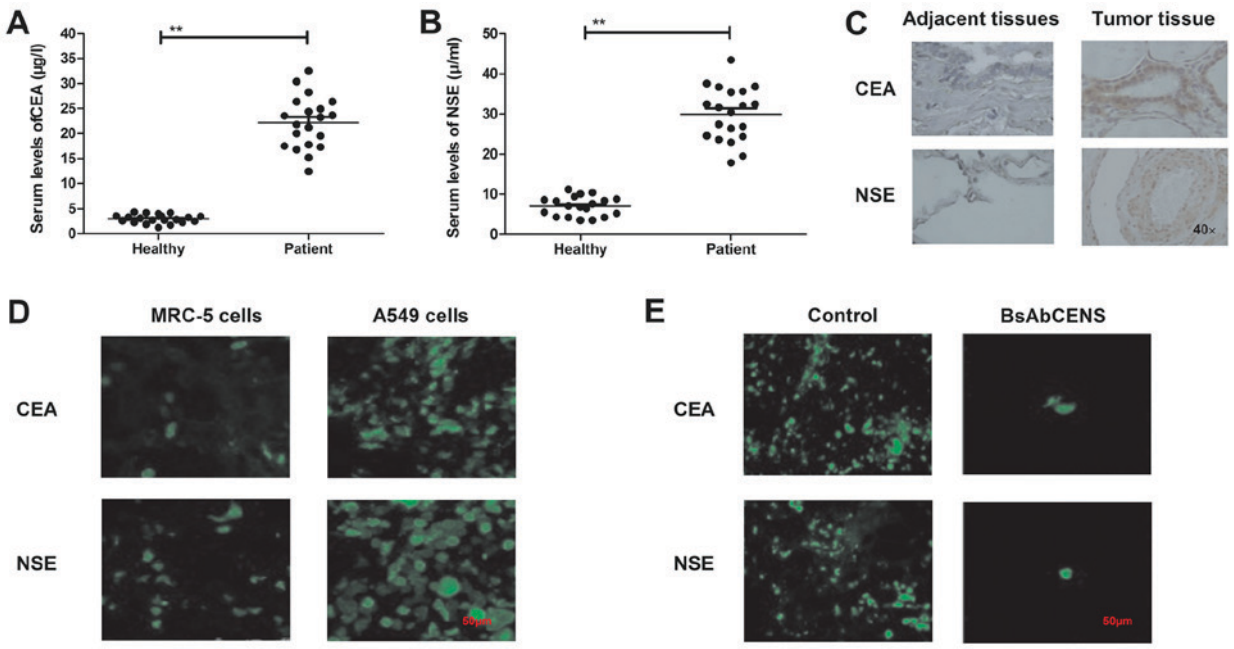

Figure 1. Analysis of the plasma expression of CEA and NSE in lung cancer tissues and normal cells. The serum levels of (A) CEA and (B) NSE were measured in patients with lung cancer and healthy individuals. (C) Representative CEA and NSE expression pictures of lung cancer tissue compared with adjacent normal tissues. Magnification, x40. (D) Immunofluorescence analysis of the expression levels of CEA and NSE in a lung cancer cell line (A549) and normal lung cell line (MRC-5). (E) The neutralization role of BsAbCENS on CEA and NSE expression levels in lung cancer cells. **P<0.01 vs. CEMRI. CEA, carcino-embryonic antigen; NSE, neuron-specific enolase; BsAbCENS, chistosan/Fe_3O_4-enclosed bispecific antibodies.

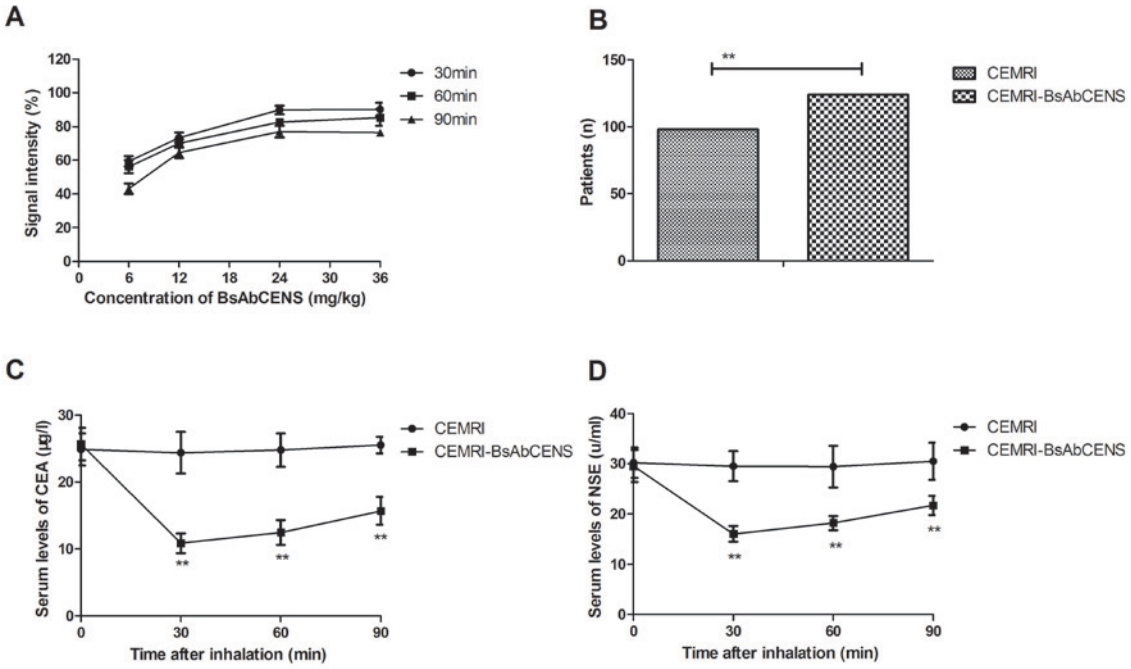

Figure 2. Efficacy of CEMRI-BsAbCENS for the diagnosis of patients with suspected lung cancer. (A) Dose determination of the signal intensity of CEMRI-BsAbCENS for clinical patients. (B) The efficacy of CEMRI-BsAbCENS and CEMRI for the diagnosis of patients with lung cancer was determined. Serum levels of (C) CEA and (D) NSE in patients with lung cancer following the inhalation of BsAbCENS. **P<0.01 vs. CEMRI. CEA, carcino-embryonic antigen; NSE, neuron-specific enolase; CEMRI, contrast-enhanced magnetic resonance imaging; BsAbCENS, chistosan/Fe_3O_4-enclosed bispecific antibodies.

inhalation (Fig. 4A). It was observed that patients diagnosed with lung cancer by CEMRI-BsAbCENS had a significantly improved survival rate compared with the mean 5-year survival rate (Fig. 4B). These results indicate that BsAbCENS increased the signal intensity for CEMRIs in patients with lung cancer.

Discussion

MRI is a type of imaging technique that is currently available for the noninvasive diagnosis of human carcinomas (29,30). CEMRI has been demonstrated to be more effective than

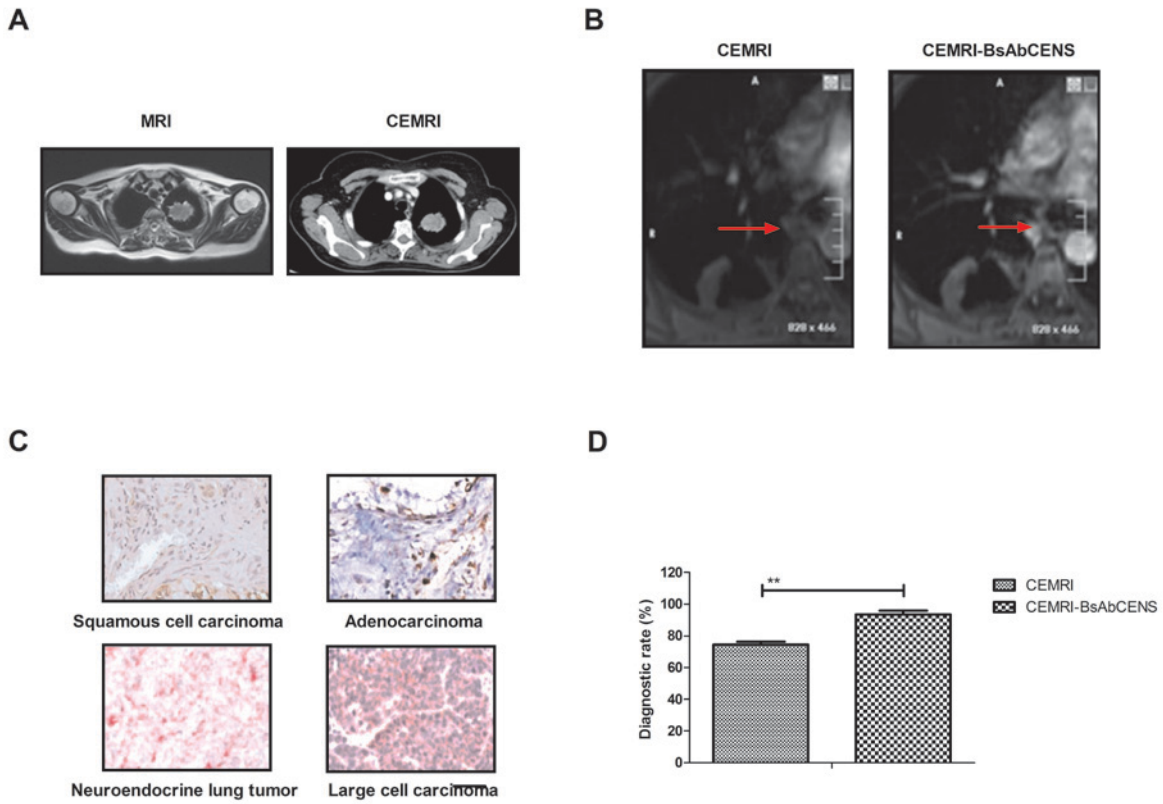

Figure 3. Histopathology analyses of the diagnostic outcomes of CEMRI-BsAbCENS for patients with lung cancer. (A) The BsAbCENS contrast agent increased the image quality generated by MRI. (B) Representative MRI images generated by CEMRI and CEMRI-BsAbCENS for patients with lung cancer. Red arrows indicate tumor location. (C) Representative histological analyses of lung tissues in patients with lung cancer. (D) The diagnostic rate of CEMRI-BsAbCENS and CEMRI for patients with suspected lung cancer. MRI, magnetic resonance imaging; CEMRI, contrast-enhanced magnetic resonance imaging; BsAbCENS, chistosan/Fe_3O_4-enclosed bispecific antibodies.

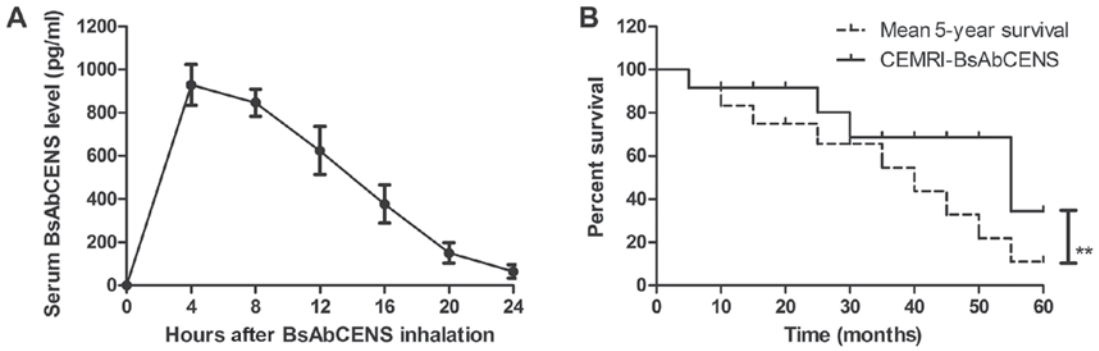

Figure 4. Pharmacodynamics of BsAbCENS in the serum of patients with lung cancer. (A) The metabolic cycle of BsAbCENS in the serum of patients with lung cancer. (B) The survival rate of patients diagnosed with lung cancer by CEMRI-BsAbCENS compared with the mean 5-year survival rate. CEMRI, contrast-enhanced magnetic resonance imaging; BsAbCENS, chistosan/Fe_3O_4-enclosed bispecific antibodies.

MRI in differentiating between melanoma and lung cancer brain metastases (31). In the present study, the diagnostic efficacy of CEMRI-BsAbCENS was further analyzed in a total of 182 patients with suspected lung cancer who had high serum levels of CEA and NSE. The results revealed that contrast agent BsAbCENS was able to bind with CEA and NSE on lung tumor cells and present a higher signal intensity than MRI in the same patients. CEMRI-BsAbCENS markedly improved sensitivity and significantly improved the diagnostic accuracy for patients with suspected lung cancer compared with MRI.

Early diagnosis of lung cancer allows for the implementation of cancer treatments that may improve the overall survival rate of patients (32-34). A previous study has demonstrated that CEMRI was able to identify patients who would benefit from bevacizumab and erlotinib treatment compared with CT based on molecular imaging for an earlier diagnosis (35). The present study reported that CEMRI-BsAbCENS diagnosed 124/182 patients with lung cancer and significantly improved the sensitivity and specificity of diagnosis. Nensa *et al* (36) have previously suggested that dynamic CEMRI parameters may act as biomarkers for the therapeutic effects of

vatalanib in patients with non-small-cell lung cancer. The present study has revealed that BsAbCENS binds with the lung cancer biomarkers CEA and NSE, which increased the signal intensity produced by MRI. Lung cancer patients had a higher survival rate following diagnosis by CEMRI-BsAbCENS compared with the mean 5-year survival rate, suggesting that CEMRI-BsAbCENS has a potential application for the early diagnosis of lung cancer. However, patients received different anti-cancer treatments, which may have affected survival rate, thus making it a limitation of this study.

Serum CEA is higher in patients with small cell lung cancer and non-small-cell lung cancer and has been used to improve the diagnostic sensitivity for human lung cancer (20,37). The clinical use of NSE has significantly improved the sensitivity and specificity of lung cancer diagnosis and may be useful for pathological typing (21). The combination of CEA and NSE may be an effective clinical confirmation and exclusive diagnostic indictor of meningeal carcinomatosis in lung cancer (38). In the present study, it was observed that BsAbCENS targeted CEA and NSE on lung cancer cells. A previous study has indicated that MRI contrast agent was able to detect an early stage glioma (39). In addition, fibronectin-targeting contrast agent in combination with MRI may detect breast cancer micrometastases (40). The present study revealed that BsAbCENS, when used with MRI, significantly improved the diagnostic accuracy and sensitivity for patients with lung cancer. Furthermore, contrast-agent MRI targeting of CA1 may produce high-resolution MR molecular imaging of human lung adenocarcinoma A549 cells (41). The results of the present study revealed that CEMRI-BsAbCENS was more effective than MRI for the diagnosis of patients with lung cancer. However, stratification for NSE ≥15 μ/ml was not conducted for patients with suspected lung cancer, which indicates a potential bias in the analysis.

In conclusion, the present study has described a novel approach for improved diagnostic accuracy of patients with lung cancer within a clinical setting, using MRI images with a targeting contrast agent. The effect of this approach on the overall survival of patients with lung cancer has also been discussed. These results suggest that CEMRI-BsAbCENS may present a significant clinical advantage to clinicians diagnosing patients with suspected lung cancer. However, further research should be conducted with a larger cohort to confirm these findings.

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Availability of data and materials

The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JG, LL, Xl and RG performed the experiments and BZ designed and analyzed the data of the present study.

Ethics approval and consent to participate

The experiments in the present study were performed in accordance with the recommendations of the Guide for the Care and Use of Clinical Study of China (approval no. BUCMT20070612M25). The present study was approved by ethics committee of Shandong Medical Imaging Research Institute (Jinan, China) and all patients provided written informed consent prior to their inclusion within the present study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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