Contrast-enhanced computerized tomography combined with a targeted nanoparticle contrast agent for screening for early-phase non-small cell lung cancer

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Abstract. Non-small cell lung cancer (NSCLC) is a major cause of morbidity and mortality, and patients with NSCLC are frequently diagnosed at an advanced stage. This is primarily due to a lack of advanced and sensitive protocols for the detection of early stage NSCLC. Therefore, methods for the accurate diagnosis of early stage NSCLC are urgently required to improve survival rates. The present study investigated the use of contrast-enhanced computerized tomography (CECT) combined with a targeted nanoparticle contrast agent (TNCA) to diagnose early-stage NSCLC in a mice xenograft model. The TNCA used was lenvatinib, a multi-target tyrosine kinase inhibitor that inhibits vascular endothelial growth factor receptor 1-3, fibroblast growth factor receptor 1-4, platelet-derived growth factor receptor β, proto-oncogene tyrosine-protein kinase receptor Ret and mast/stem cell growth factor receptor Kit. Xenograft NSCLC mice were established and used to analyze the efficacy of CECT-TNCA compared with CT scanning alone. The TNCA was inhaled with the use of an atomizer. The results demonstrated that CECT-TNCA improved the sensitivity of the diagnosis of early stage NSCLC. In addition, imaging using the TNCA enabled the visualization of nodules in the lung in mice with early stage NSCLC. In addition, lung nodule signal enhancement was increased in CECT-TNCA compared with CT, suggesting a high accurate accumulation of the TNCA in tumor nodules. Mice diagnosed with early stage NSCLC exhibited a higher eradication rate of NSCLC after treatment with cisplatin compared with mice with advanced stage NSCLC. These data indicate that the sensitivity and accuracy of CT imaging for the diagnosis of early stage NSCLC was improved through combination with the liposome-encapsulated TNCA.

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Key words: non-small cell lung cancer, contrast-enhanced computerized tomography, nanoscale microbubble, liposome-encapsulated lenvatinib

Introduction

Non-small cell lung cancer (NSCLC) is a major cause of morbidity and mortality (1,2). DeCotiis *et al* (3) reported that NSCLC is typically an aggressive cancer. The occurrence of NSCLC has increased in developing countries due to rising industrial pollution (4,5). The types of NSCLC include adenocarcinoma, large cell carcinoma and squamous cell carcinoma, which together account for ~80% of all lung cancer cases (6,7). Despite advancements in the therapeutic regimens for lung cancer, the survival rate of patients with NSCLC remains poor (8-10). This is due to NSCLC typically being diagnosed at an advanced stage (11,12). In addition, the aggressiveness of NSCLC contributes to tumor recurrence and poor survival rates for patients (13,14). These reports suggest that the early diagnosis of NSCLC would be a best way to improve the survival of patients with NSCLC.

The majority of patients with NSCLC have an advanced stage tumor at the time of diagnosis (15). In order to increase the efficacy of anticancer drugs and decrease mortality rates, the focus of lung cancer management has shifted to early diagnosis and personalized anticancer therapy (16,17). Hence, different diagnostics tools for patients with NSCLC have been explored (11,18). Clinically, computerized tomography (CT) scanning is frequently used to diagnose NSCLC (19). Subsequently, histopathology is applied to confirm the final diagnosis (20). Aberle *et al* (21) reported that CT screening had altered the landscape of lung-cancer screening and decreased lung cancer-associated mortalities by 20%, indicating the potential efficacy of screening for early stage lung cancer using CT imaging. However, CT used alone is not sensitive enough for the detection of early stage NSCLC (22,23). Seigneurin *et al* indicated that the presence of positron emission tomography (PET) in the work-up protocol is associated with higher recall rates, detection rates and positive predictive values of CT screening for lung cancer (24).

The aforementioned diagnostic methods typically diagnose patients with NSCLC at an advanced clinical stage, losing the optimal time frame for treatment and thus shortening survival times. Therefore, modified and optimized CT screening techniques should be developed for the diagnosis of early stage NSCLC. Novel imaging techniques could enhance the sensitivity of the detection of early stage tumor morphology. A previous study has evaluated the application of dynamic contrast-enhanced CT imaging in the study of the differentiation of benign and malignant tumors, observed by tumor vessel and permeability nodule perfusion (25). However, conventional contrast agents present lower efficacy for tumor analysis due to their rapid diffusion away from the lungs (26). Furthermore, previous reports have demonstrated that iodinated contrast agents are less sensitive to changes in cell morphology (27,28).

Nanoparticle-based imaging contrast agents exhibit potential in diagnosing early-stage cancer through providing a more accurate and sensitive detection of tumor nodules (29,30). Cho *et al* (31) investigated inorganic nanoparticles containing semiconductor quantum dots, iron oxide and gold, which revealed their potential for use as contrast agents combined with CT for diagnostics.

The present study investigated the use of a nanoscale microbubble contrast agent for contrast-enhanced (CE) CT to detect early stage NSCLC. This highlighted the potential application of CECT-targeted nanoparticle contrast agent (TNCA) imaging in the diagnosis of NSCLC. The results revealed the advantages of CECT-TNCA compared with CT alone in the early diagnosis and final confirmation of NSCLC. CECT-TNCA, with the contrast agent inhaled by nebulization, led to lesions being augmented and amplified in the lung during imaging, resulting in a reliable and sensitive assessment for the clinical diagnosis of NSCLC.

Materials and methods

Ethics statement. The present study was performed in accordance with the recommendations for the Guide for the Care and Use of Laboratory Animals of The First Hospital of Shijiazhuang (Shijiazhuang, China), and all animal work was approved by the Ethics Committee of The First Hospital of Shijiazhuang. All surgery and euthanasia were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Scanning protocol. A CECT diagnosis system (MX4000, Philips Medical Systems, Inc., Bothell, WA, USA) was used to analyze the lungs using a preprogrammed setting, which was optimized to obtain the best image. Details of the settings used are described in previous study (32).

TNCA. A novel liposome-encapsulated contrast agent comprising lenvatinib-bound nanoparticles was used in the present study. The lenvatinib was bound to superparamagnetic iron oxide nanoparticles via a covalent bond, as described in a previous study (33). The route of administration for the TNCA or Optison™ (GE Healthcare Life Sciences, Little Chalfont, UK; used as a control) was inhalation using an atomizer. The microbubbles containing lenvatinib could reach the lesion through the pulmonary circulation due to their small diameter. After 30 min, the TNCA could be visualized using the CECT system. No side effects were observed from the nanoparticle-lenvatinib contrast agent.

Table I. Characteristics of mice with NSCLC.

Characteristic	Gender, no. of mice	
	Male	Female
Number	60	60
NSCLC	60	60
Adenocarcinoma	20	20
Large cell carcinoma	20	20
Squamous cell carcinoma	20	20

All mice were 6-8 weeks old. NSCLC, non-small cell lung cancer; CECT, contrast-enhanced computerized tomography; TNCA, targeted nanoparticle contrast agent.

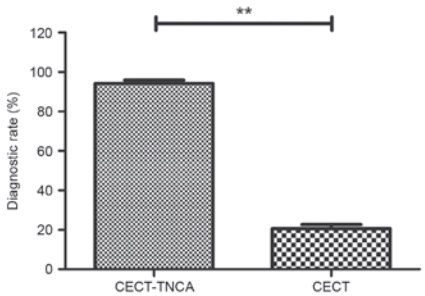

Figure 1. Mice with non-small cell lung cancer diagnosed by CECT-TNCA or CECT. **P<0.01. CECT, contrast-enhanced computerized tomography; TNCA, targeted nanoparticle contrast agent.

Animal study. NSCLC tumors were established in mice as described previously (34). Briefly, C57BL/6 mice (age, 6-8 weeks) were purchased from Shanghai Laboratory Animal Co., Ltd. (Shanghai, China) and underwent intranasal infection with NSCLC cells (H358, NCI-H1755 or NCI-H661). NSCLC mice included adenocarcinoma, large cell carcinoma and squamous cell carcinoma (n=40 in each group). These mice were used for the imaging study 7 days after infection. All mice were housed in a temperature-controlled facility at 23±1˚C and relative humidity of 50±5% with a 12-h light/dark cycle. All animals had free access to food and water. A total of 120 animals (60 male, 60 female; tumor free, n=34; tumor-bearing, n=86) were used to examine the efficacy of CECT-TNCA. Subsequently, the liposomal contrast agent (1,400 mg/kg) was inhaled for 20 min via an atomizer. Post-contrast scans were then conducted immediately (0 h), and 72 and 120 h after inhalation of the liposomal contrast agent. The experimental animals were then sacrificed, and 4-μm tumor specimens were fixed in 10% formaldehyde for 15 min at 37˚C and underwent hematoxylin and eosin staining (2 h at 37˚C) to confirm the tumor phenotype and morphology using a light microscope. All mice with early or late phase NSCLC were treated with cisplatin (1.2 mg/day) for a total of 10 days. Ten mice in each group were housed to observe the survival rate in 120 days.

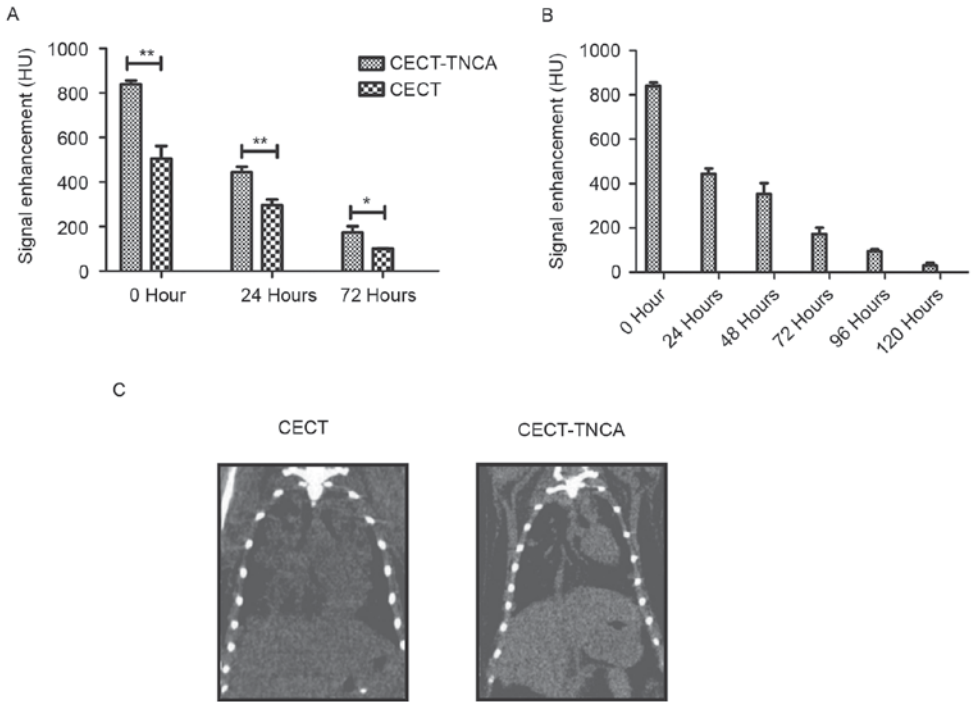

Figure 2. CECT-TNCA promoted signal enhancement, which was associated with tumor diameter. (A) Signal enhancement was significantly enhanced in lesions imaged with CECT-TNCA compared with CECT. (B) Signal enhancement over time with used of the TNCA. (C) Dynamic analysis of enhanced signaling after inhalation of the TNCA. *P<0.05, **P<0.01. CECT, contrast-enhanced computerized tomography; TNCA, targeted nanoparticle contrast agent.

Image data analysis. Data from the CECT-TNCA images was analyzed using the CECT system, including the volume of the tumors.

Statistical analysis. All data are presented as the mean ± standard deviation of triplicate experiments. Data was analyzed by SPSS 19.0 software (IBM Corp., Armonk, NY, USA) using one-way analysis of variance with Tukey's multiple comparison test. The Kaplan-Meier estimator was used to produce survival curves for the mice over the 120-day long treatment period. P<0.05 was considered to indicate a statistically significant difference.

Results

CECT-TNCA improves the sensitivity of the diagnosis of early stage NSCLC. A total of 120 mice with early stage NSCLC (adenocarcinoma, large cell carcinoma and squamous cell carcinoma; n=40/group) were used to analyze the efficacy of CECT-TNCA on mice with early stage NSCLC. Tumor metastasis was not observed in any of the mice. CECT-TNCA identified significantly more of the mice with NSCLC compared with CECT (32/120 vs. 7/120, respectively; P<0.001; Fig. 1). The characteristics of mice with NSCLC are detailed in Table I. These data suggest that, compared with CECT, CECT-TNCA is more sensitive for the diagnosis of early NSCLC.

CECT-TNCA improves the signal intensity fed back by lesions. In order to analyze the efficacy of CECT-TNCA, signal intensity was detected after administration of the liposomal contrast agent. This revealed that the nanoscale microbubble contrast agent significantly enhanced the signal intensity compared with CECT (P<0.05; Fig. 2A). The nanoscale microbubble contrast agent was cleared from systemic circulation in ~120 h (Fig. 2B). Dynamic analysis of lung tumor nodules revealed an enhanced signal after inhalation of the TNCA (Fig. 2C). These results suggest that the TNCA significantly improved signal intensity in lung tumor nodules.

Histological analysis of the phenotype of NSCLC. The phenotype of NSCLC was further analyzed by histological analysis (Fig. 3). Adenocarcinoma, large cell carcinoma and squamous cell carcinoma were identified by histological analysis. In addition, mice diagnosed by CECT-TNCA and CT were compared (Fig. 4). CECT-TNCA identified a higher number of mice with NSCLC compared with CT. These results highlight the accuracy of CECT-TNCA in diagnosing NSCLC.

Efficacy of CECT-TNCA. The pulmonary tumor nodules were further analyzed by CECT-TNCA and CECT, with results revealing a positive association between diameter and signal intensity (Fig. 5). Imaging analysis included NSCLC volume and fractional blood volume as a function of nodule diameter. In addition, signal intensity fed back by lesions in the NSCLC nodules was improved after administration of the targeted nanoscale microbubble contrast agent. These factors enabled the visualization of tiny tumor lesions in the lung (Fig. 6). Furthermore, the longitudinal aspect of CECT-TNCA allowed for the imaging of early-stage NSCLC tumors in mice (Fig. 7). In addition, the data demonstrated that survival after treatment was improved in mice with early-stage NSCLC compared with late-stage NSCLC (Fig. 8). These results indicate that CECT-TNCA-diagnosed early-stage tumors exhibited relatively higher signal enhancement compared with CT tumors in xenograft mice.

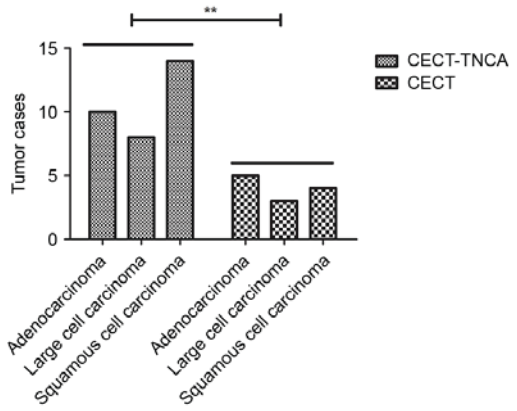

Figure 3. Histological analysis of the phenotype of NSCLC. Hematoxylin and eosin staining was performed to analyze the phenotype of NSCLC. Magnificiation, x400. **P<0.01 CECT-TNCA vs. CECT. NSCLC, non-small cell lung cancer; CECT, contrast-enhanced computerized tomography; TNCA, targeted nanoparticle contrast agent.

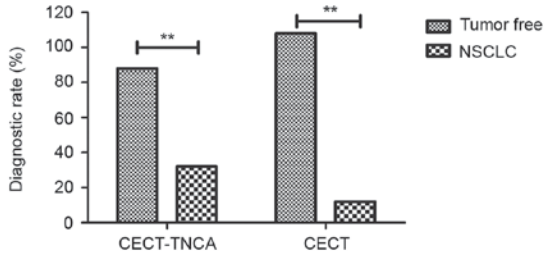

Figure 4. Analysis of the diagnostic rate of NSCLC from CECT-TNCA and CECT. **P<0.01. NSCLC, non-small cell lung cancer; CECT, contrast-enhanced computerized tomography; TNCA, targeted nanoparticle contrast agent.

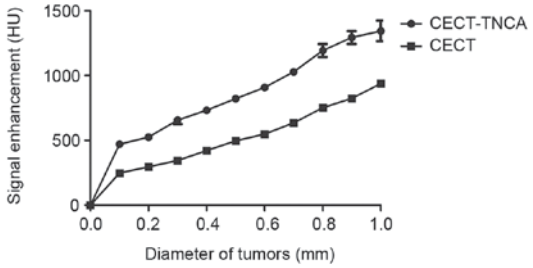

Figure 5. Signal enhancement compared with tumor diameter from CECT-TNCA and CECT. CECT, contrast-enhanced computerized tomography; TNCA, targeted nanoparticle contrast agent.

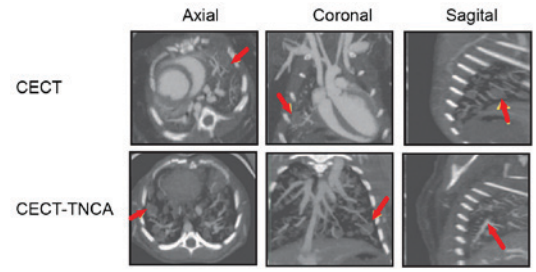

Figure 6. Observation of small tumor lesions in the lung by CECT-TNCA and CECT. Tumor lesions are indicated by an arrow. Magnification, x10. CECT, contrast-enhanced computerized tomography; TNCA, targeted nanoparticle contrast agent.

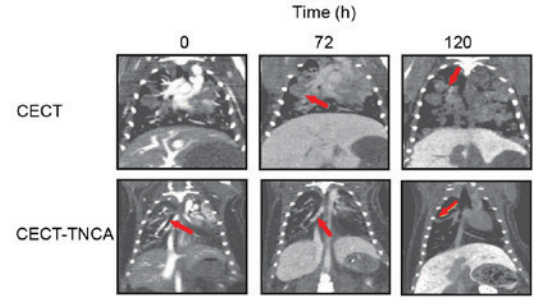

Figure 7. Representative CECT-TNCA and CECT images of mice with non-small cell lung cancer at 0, 72 and 120 h after inhalation of the TNCA. CECT, contrast-enhanced computerized tomography; TNCA, targeted nanoparticle contrast agent.

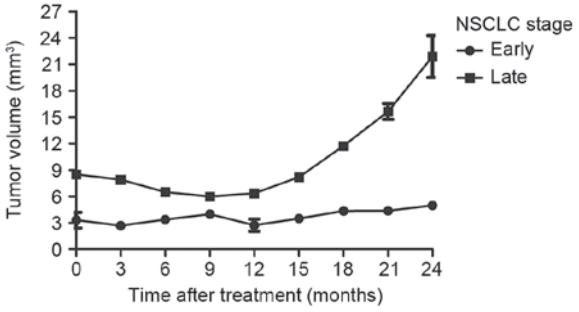

Figure 8. Early stage diagnosis of NSCLC by CECT-TNCA improved mice survival compared with late stage diagnosis of NSCLC by CECT. NSCLC, non-small cell lung cancer; CECT, contrast-enhanced computerized tomography; TNCA, targeted nanoparticle contrast agent.

Discussion

NSCLC is one of the most common of types of respiratory cancer and a leading cause of cancer-associated mortality worldwide (35). The incidence of NSCLC and the number of NSCLC-associated mortalities is growing (36). Notably, the majority of newly diagnosed patients with NSCLC are already in a late phase, which decreases the probability of recovery and shortens survival time (37). Therapeutic regimens for advanced NSCLC include targeted intervention, chemotherapy, radiotherapy and immunotherapy (38).

Late-phase NSCLC is more aggressive, contributing to a poor survival rate (39,40). Patients with advanced stage NSCLC more frequently exhibit drug resistance and side effects (41). Therefore, diagnosis protocols for early stage NSCLC are required to improve patient survival. The early detection of NSCLC could improve patient survival due to earlier surgery, conventional treatments and comprehensive therapy (38). Clinically, the following imaging technologies are the most commonly used to diagnose tumors: Ultrasound, X-ray, CT scanning, PET scanning and magnetic resonance imaging (MRI) (42). CT scanning is relatively economic and accurate compared with the aforementioned imaging technologies (43,44). CT scanning and MRI are the most commonly used techniques to provide high spatial resolution imaging to assess tumors. Despite PET and single positron emission computerized tomography providing high-contrast sensitivity for NSCLC diagnosis, they provide a relatively low spatial

resolution (45). Although CT scanning provides high spatial resolution, it is not efficient at diagnosing patients with early stage lung cancer (46,47).

The present study investigated a comprehensive approach of CECT combined with a TNCA in mice, in order to improve the accuracy of early stage NSCLC diagnosis. Lenvatinib encapsulated by liposomes was used as the TNCA. Lenvatinib is a multi-target tyrosine kinase inhibitor of vascular endothelial growth factor receptor (VEGFR) 1-3, fibroblast growth factor receptor (FGFR) 1-4, platelet-derived growth factor receptor (PDGFR) β, proto-oncogene tyrosine-protein kinase receptor Ret (Ret) and mast/stem cell growth factor receptor Kit (Kit). VEGF1-3, FGFR1-4, PDGFR-β, Ret and Kit-mediated angiogenesis have been identified as key factors in the development of human cancer (48,49). Lenvatinib has potent antitumor activity against a number of human tumors (50). A previous report has suggested targeting the receptor of lenvatinib in NSCLC (51). The results of the present study demonstrated that liposome-encapsulated lenvatinib has a potential application as a TNCA to improve the accuracy of early-stage NSCLC diagnosis. This technique enhanced the signal intensity in lesions in the lung, improving the spatial resolution of CECT.

Previously, liposomal and iodinated contrast agents have provided methods to detect solid tumors (52,53). The feasibility of spectral CT imaging for the detection of tumors with target-based contrast agents was evidenced in a previous study. Contrast agents have improved CT diagnosis in terms of image quality, as observed in a prospective randomized trial (54). However, the development of a non-invasive assay for the accurate diagnosis of NSCLC remains a challenge. The present study investigated the efficacy of a liposome-encapsulated lenvatinib TNCA for the diagnosis of early stage NSCLC in a xenograft mice model. The results revealed that CECT-TNCA-diagnosed early stage NSCLC tumors exhibited increased signal enhancement compared with CT-diagnosed tumors. This suggests that the liposome-encapsulated targeted contrast agent enables high-resolution imaging of early phase NSCLC.

In conclusion, the current study provided insights into improving CT imaging resolution using a liposome-encapsulated targeted contrast agent. The preclinical application of thus imaging procedure would provide novel opportunities to assess the efficacy of early-stage NSCLC diagnosis and determine whether nanoparticles exhibit absorbability without affecting the respiratory system. The results of the present study indicate that CECT-TNCA may be of significant value in the diagnosis of NSCLC. Further studies are required to validate these results.

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