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CHEST IMAGING

ORIGINAL ARTICLE

Establishment of a large animal model for research on transbronchial arterial intervention for lung cancer

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PURPOSE
We aimed to evaluate whether bronchial artery can supply a percutaneously inoculated canine transmissible venereal tumor (CTVT) in a lung tumor model.

METHODS
Fresh CTVT tissue blocks were percutaneously inoculated into unilateral or bilateral lungs of six immunosuppressed dogs at the mid zone of the middle or lower lobe. Tumor growth was monitored by computed tomography (CT). Ten weeks after inoculation, pulmonary arterial digital subtraction angiography (DSA), bronchial arterial DSA, transpulmonary arterial contrast-enhanced multislice CT, transbronchial arterial contrast-enhanced multislice CT (BA-MSCT), and transpulmonary arterial lipiodol multislice CT were performed.

RESULTS
Tumor growth was seen in all 10 inoculated sites, with a maximum diameter of 2.734±0.138 cm at 10th week. Bronchial arterial blood supply was evident in 9 nodules on DSA, and was equivocal in one which was later demonstrated on BA-MSCT. No obvious pulmonary arterial blood supply was observed in any of the nodules. Lipiodol deposition was displayed in two of the small distant metastases, which indicated that pulmonary artery was involved in the supply of the metastases.

CONCLUSION
Our results demonstrated bronchial arterial blood supply in this new lung cancer model. This model may be used in further research on transbronchial arterial intervention for lung cancer.

Bronchial arterial infusion chemotherapy (BAI) for lung cancer was introduced into clinical practice 50 years ago (1–3). Theoretically, better reductions in tumor size and symptoms, and less adverse effects of anticancer drugs could be achieved with direct infusion of high-density chemotherapeutics into tumors. However, BAI for lung cancer is not widely accepted. In the last two decades, only a few small case series were published in the English literature showing favorable results (4–9). This may be explained by several reasons: the outcomes have not been confirmed, severe complications have been reported (10, 11), the pharmacokinetics of BAI has not been fully understood, the indications and the treatment protocols have not been defined (4, 12). In the near future, the role of BAI or other transbronchial arterial therapy in the combined treatment of lung cancer may be reappraised, given the poor 5-year survival rate of less than 17% despite improvements in therapeutic management (13).

Unfortunately, there is currently no large animal lung cancer model for fundamental research on transbronchial arterial therapy. In 2002, Ahrar et al. (14) developed a canine lung tumor model by intra-arterial or percutaneous inoculation of canine transmissible venereal tumor (CTVT) fragments, which was later used for study on percutaneous radiofrequency ablation (15). It is well known that metastatic lung cancer receives blood supply from both pulmonary artery and bronchial artery, with peripheral tumors having a predominant pulmonary circulation and central tumors having a predominant bronchial circulation (16). Our study goal is to evaluate the blood supply of this large animal lung tumor model.

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Methods

Animal model

Animal experiments were approved by the Animal Care Committee of our university (IACUC-20121014-05). Animal model establishment was based on the protocol by Ahrar et al. (14). Fresh CTVT samples were taken from pre-existing tumors in the paraspinal muscles of a beagle donor dog inoculated 8 weeks before and cut into small blocks approximately 1.5–2.0 mm in diameter and placed in iced Hanks medium (Westang biotechnology). Six beagle dogs weighing 10.0–13.5 kg (mean, 10.56±1.86 kg) were obtained and raised at Shanghai Laboratory Animal Research Center. To promote tumor establishment and growth, all dogs were treated with oral 10 mg/kg cyclosporin (Huadong Medicine) twice daily for 14 days beginning 7 days before inoculation and once daily thereafter. Before inoculation or imaging procedure, the dogs were anesthetized with an intraperitoneal injection of 3% 1 mL/kg pentobarbital (Westang Biotechnology). Six dogs underwent computed tomography (CT)-guided percutaneous unilateral (first two dogs) or bilateral (next four dogs) lung inoculation at the mid zone of their middle or lower lobes. Spiral CT (LightSpeed VCT, General Electric) images were obtained to plan the inoculation. After selecting the puncture site as well as needle course and ultimate location of inoculation, a 16 G guiding needle (Invatec) was advanced into the lung parenchyma. CT imaging was repeated after each needle placement to confirm the appropriate location of the needle tip. Through the needle, two to three pieces of fresh tumor block were pushed into lung parenchyma and followed by gelfoam (Zhonghua Pharmaceutical Co.) strips. All inoculations were performed within two hours after harvesting the tumor. The dogs were then closely monitored and housed at Shanghai Laboratory Animal Research Center.

To observe the tumor growth, chest CT scan was performed on all dogs immediately, the 4th, 6th, 8th, and 10th week after inoculation. For CT imaging, the dogs typically were placed in the supine position and images were acquired in helical mode at 120 kV, 200 mA, pitch 0.516 and 0.625 mm slice thickness.

Main points

- In this experiment, we established a large animal model for research on transbronchial arterial intervention for lung cancer.
- Our results indicate that the current model may potentially be used for fundamental research regarding transbronchial arterial therapy for lung cancer.
- Potential applications include studying the intratumor pharmacokinetics after bronchial arterial infusion chemotherapy, refining intralesion drug delivery techniques and defining treatment protocols. In addition, this model may also be used to help evaluate treatment outcomes of transbronchial arterial administration of new therapeutic agents.

Evaluation of blood supply

Ten weeks after inoculation, the dogs were sedated with an intramuscular injection of 0.5 mg/kg medetomidine (Westang Biotechnology) and a subcutaneous injection of 0.4 mg/kg atropine sulfate (Westang Biotechnology). Once they were sedated, surgical anesthesia was induced and maintained with 2–3 L/min oxygen and 2%–5% isoflurane (Westang Biotechnology). Unilateral femoral artery and vein were surgically dissected, and inserted with 5 F sheaths (Terumo). Pulmonary arterial digital subtraction angiography (PA-DSA), bronchial arterial digital subtraction angiography (BA-DSA), transpulmonary arterial contrast enhanced multislice CT (PA-MSCT), transbronchial arterial contrast-enhanced multislice CT (BA-MSCT), and transpulmonary arterial lipiodol multislice CT (PA-L-MSCT) were then performed. Below are some details of the work.

PA-DSA: Through the femoral vein, a 5 F Cobra or Pigtail catheter (Terumo) was introduced into the pulmonary artery and DSA was performed at an injection rate of 5 mL/s for a total volume of 15 mL Iopromide (Ultravist 370, Bayer) under DSA machine (Infinix, Toshiba). The catheter was then flushed with heparinized saline and sealed with a luer-cock.

BA-DSA: Through the femoral artery, a 5F Cobra catheter (Terumo) was introduced into the thoracic aorta. To locate the bronchial arteries, bilateral intercostal arteries at the level between T5 and T8 were catheterized and DSA was performed at an injection rate of 1 mL/s for a total volume of 5 mL Iopromide (Ultravist 370, Bayer). A coaxial 3 F microcatheter (Marguerite, GMA) was manipulated 1–2 cm into the bronchial arteries once they were found, and BA-DSA was performed at an injection rate of 1 mL/s for a total volume of 5 mL. The microcatheter was then left in unilateral bronchial artery, flushed with heparinized saline and sealed.

PA-MSCT: 30 minutes after DSA, dogs were moved to CT room. Plain chest CT was first performed covering from thoracic inlet to the diaphragm. The catheter left in pulmonary artery was connected to injector, and PA-MSCT was done in three dogs at an injection rate of 5 mL/s for a total volume of 15 mL contrast (Ultravist 370, Bayer).

BA-MSCT: The microcatheter was connected to injector, and BA-MSCT was started when 5 mL of contrast had been injected at a rate of 1 mL/s.

PA-L-MSCT: In three dogs in which PA-MSCT was not performed, 5 mL of lipiodol (Guerbet) was slowly injected through the catheter left in pulmonary artery, afterwards CT scan was performed.

All dogs were killed by exsanguination under deep anesthesia. The dissected lung tumors were fixed for pathological examination by immersion in 10% buffered formalin (Westang biotechnology). Tissues then underwent standard histologic processing and 0.4~0.6 μm paraffin sections were stained with hematoxylin and eosin (Westang biotechnology).

Measurements and analysis

Lung tumor growth was recorded at each time by measuring the maximum diameter of the tumors. The criterion of blood supply on DSA was vessel entering into tumor and/or staining of the tumor. The criteria of blood supply on CT was presence of chaotic vessels, accumulation of contrast within tumor, or lipiodol accumulation in tumor.

Results

Inoculation was successfully performed in all six dogs, resulting in 10–20 mm patchy, irregular, well-defined hyperdensities in the inoculated lung fields. Minor pneumothorax occurred in four dogs, and minor pneumorrhagia occurred in three dogs. All dogs were in good general condition throughout the experimental period. At 4th week, no growth could be seen. At 6th week, unilateral or bilateral solitary pulmonary nodules with maximum diameter of 1.716±0.102 cm could be seen at the inoculated sites in all the dogs, which grew rapidly thereafter reaching maximum diameter of 2.392±0.076 cm at 8th week and 2.734±0.138 cm at 10th week (Figs. 1–3). At 10th week, multiple tiny distal subpleural nodules <1.0 cm in diameter were observed

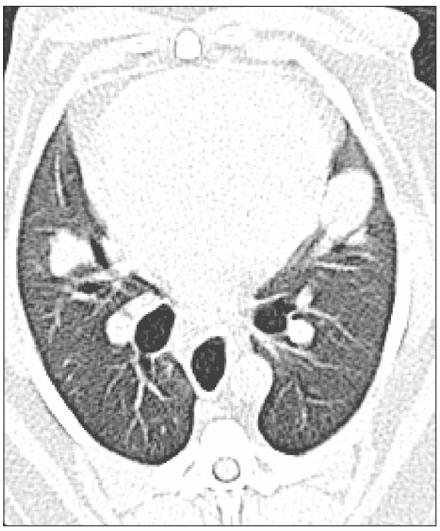

Figure 1. Axial CT image shows lung canine transmissible venereal tumor (CTVT) growth at 6th week.

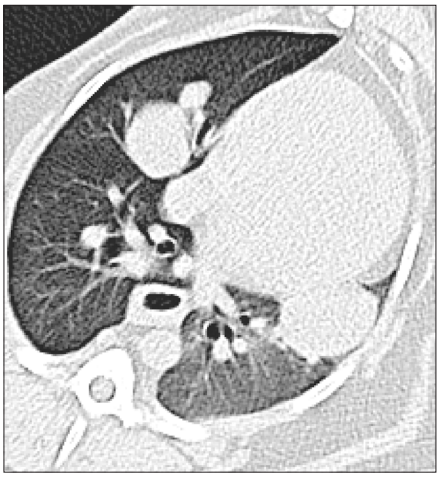

Figure 2. Axial CT image shows lung CTVT growth at 8th week.

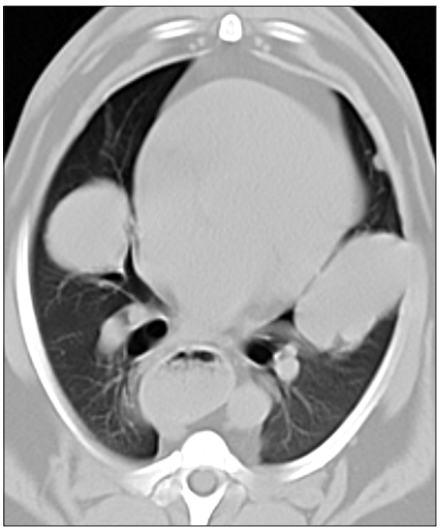

Figure 3. Axial CT image shows lung CTVT growth at 10th week.

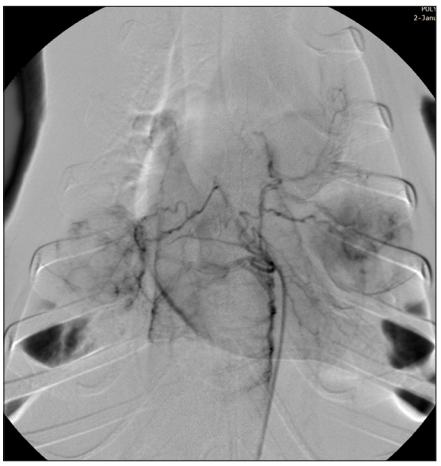

Figure 4. Digital subtraction angiography (DSA) image shows a common bronchial artery supplying bilateral tumor nodules with scattered tumor stain (same dog as in Fig. 1).

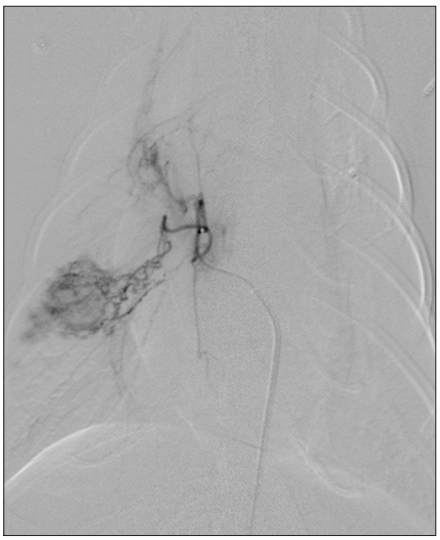

Figure 5. DSA image shows dilated right bronchial artery supplying the right tumor nodule.

in two dogs, and right moderate hydrothorax developed in one dog.

Totally nine bronchial arteries were located, all originating from the intercostal arteries at the level between T6 and T8 (mostly at the mid-level between T6 and T7). Five right bronchial arteries, two left bronchial arteries and one common bronchial artery originated from right intercostal arteries. Only one left bronchial artery originated from left intercostal artery. In three of the nine bronchial arteries, superselective catheterization with microcatheter failed, and angiography was performed with the microcatheter in the intercostal arteries. Bronchial arterial blood supply was evident in nine nodules with mild to moderate tumor stain on BA-DSA (Figs. 4, 5). In one nodule,

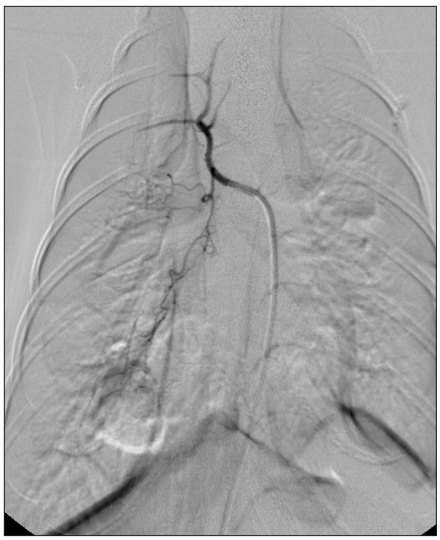

Figure 6. DSA image shows the right bronchial artery coursing towards the tumor nodule at the right low lobe. No discernible tumor stain could be seen.

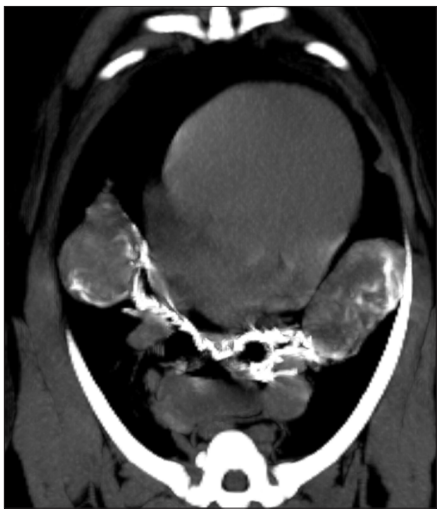

Figure 7. Oblique axial maximum intensity projection (MIP) reconstruction of transbronchial arterial contrast-enhanced multislice CT (BA-MSCT) shows that the dilated common bronchial artery led to bilateral tumor nodules with accumulation of contrast within the nodules (same dog as in Fig. 1).

no discernible tumor stain could be seen, although the bronchial artery coursed towards it (Fig. 6). BA-MSCT was successful in five of six dogs, due to the dislodgement of microcatheter into thoracic aorta in one dog, which displayed chaotic blood vessels and contrast within the four nodules including the one with no tumor stain on BA-DSA (Figs. 7–9).

In all six dogs, pulmonary arteries were successfully catheterized, but neither PA-DSA nor PA-MSCT showed evident blood supply of the nodules (Figs. 10–12). PA-L-

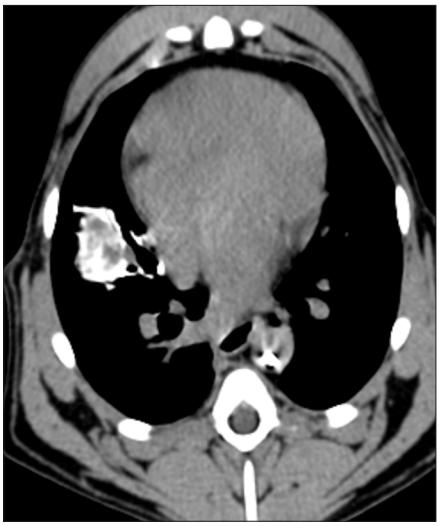

Figure 8. Axial image of BA-MSCT shows accumulation of contrast within the nodule (same dog as in Fig. 5).

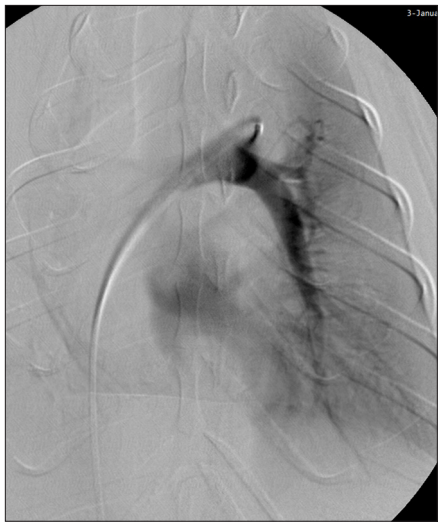

Figure 10. DSA image of the left pulmonary artery shows filling defect at left nodule area (same dog as in Fig. 1).

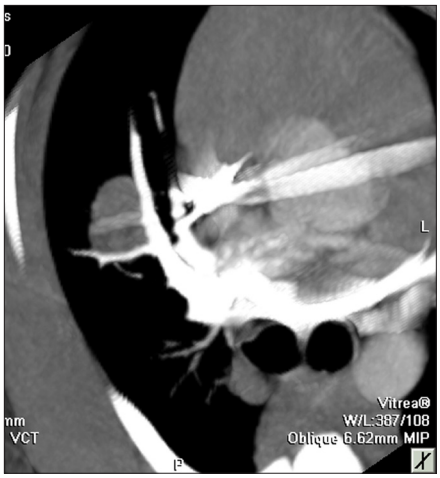

Figure 12. Oblique axial MIP reconstruction of transpulmonary arterial contrast-enhanced multislice CT (PA-MSCT) shows no obvious accumulation of contrast within the nodule (same dog as in Fig. 5).

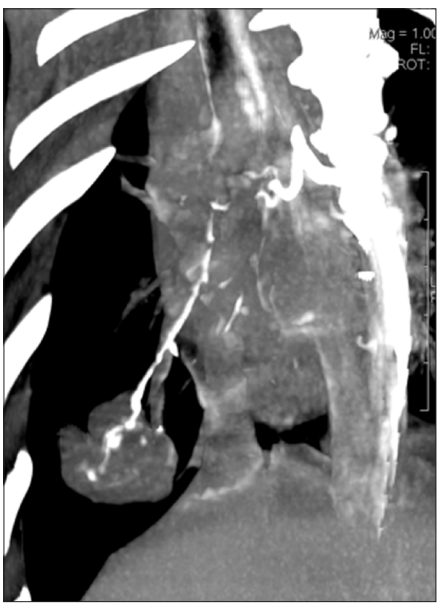

Figure 9. Oblique coronal MIP reconstruction of BA-MSCT shows that the bronchial artery entered the nodule with scattered contrast accumulation (same dog as in Fig. 6).

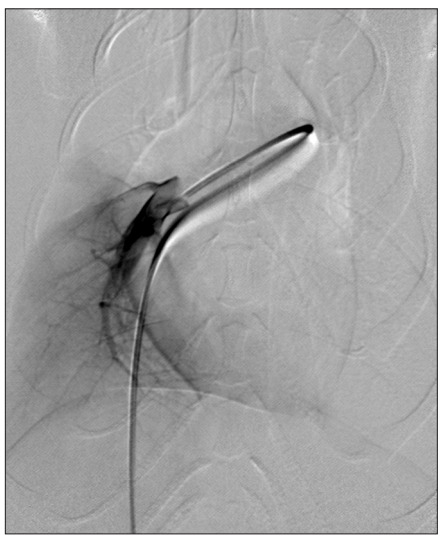

Figure 11. DSA image of the right pulmonary artery shows no obvious tumor stain (same dog as in Fig. 5).

MSCT showed no blood supply either, even though nodules were surrounded by dense lipiodol packing the adjacent normal lung tissue (Fig. 13). However, lipiodol deposition was clearly displayed in two of the small distant metastases, indicating pulmonary arterial supplement (Fig. 14).

Pathological examination was performed and confirmed the allograft CTVT tumors.

Discussion

In this experiment, we demonstrated bronchial arterial supply of this percutaneously inoculated CTVT lung tumor model. Together with the high technical feasibility of location and catheterization of the bronchial arteries, our results indicate that the current model may potentially be used for fundamental research into transbronchial arterial therapy for lung cancer. Potential applications include studying the intratumor pharmacokinetics after BAI, refining intralesion drug delivery techniques and defining treatment protocols. In addition, this model may also be used to help evaluate treatment outcomes of transbronchial arterial administration of new therapeutic agents, such as drug-eluting beads (17).

The bronchial arterial supply of the current model is within our expectations. Milne et al. (16) demonstrated the predominant bronchial circulation of centrally located metastatic lung cancer in humans and rats. In an experimental metastatic rabbit lung tumor model, tumors located near the hilus, regardless of size, were always injectable via the bronchial arteries (12). Besides, Pan et al. (18) found that intravenous injected tumors developed a pulmonary artery-derived vascular tree whereas directly injected tumors developed a bronchial artery-derived vasculature in rats. When developing the animal model, we purposefully inoculated the mid zone of the lungs in order to mimic "the more central lesion", which resulted in constant bronchial arterial supply of the nodules. In addition to the central location, tumor size should also be considered. Blood supply was evaluated at 10^{th} week after inoculation when tumors reached 2.734±0.138 cm. Yuan et al. (19) did a CT perfusion study in a small series of patients with diverse primary lung carcinomas and showed that tumor angiogenesis was somewhat size dependent, with small tumors having evident pulmonary circulation. In orthotopic model of non-small cell lung cancer in rats, Eldridge et al. (20) showed that bronchial artery perfusion parallels the growth in tumor volume, while pulmonary artery perfusion remained unchanged, so the authors concluded that bronchial artery angiogenesis drove lung tumor growth.

To our interest, although marked enhancement of the nodules could be seen on CT, selective angiography of the bronchial and pulmonary arteries was performed in only one dog, and no feeding

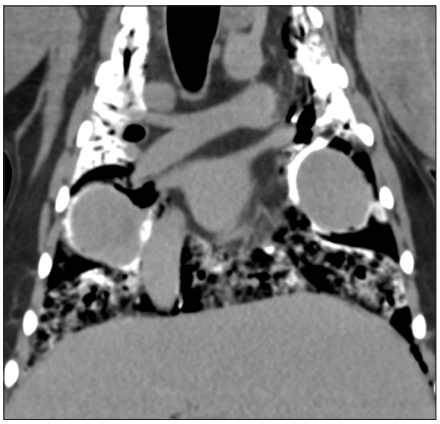

Figure 13. Oblique coronal MIP reconstruction of PA-L-MSCT shows that the nodules were surrounded with lipiodol, with no lipiodol accumulation inside (same dog as in Fig. 1).

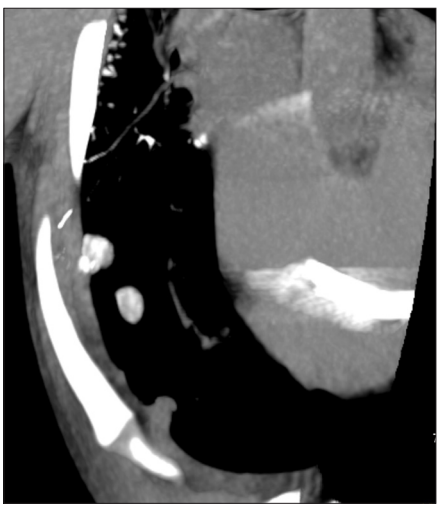

Figure 14. Oblique axial MIP reconstruction of PA-L-MSCT shows lipiodol deposition in two of the small distant metastases.

vessels to two nodules in the right apical lobe were detected in Ahrar et al. (14). Failure of detecting the feeding vessels to the hypervascular nodules by Ahrar et al. (14) may be explained by the low resolution of the two-dimensional projection of angiography. To overcome the disadvantage of 2D projection of DSA, transarterial CT was performed. BA-MSCT enabled to define the bronchial arterial blood supply of one nodule which was equivocal on BA-DSA. Ultrafast CT also enabled to perform PA-MSCT before contrast entered into systemic circulation. Lipiodol was reported to stay inside tumor tissues (21). PA-L-MSCT was later adopted, because it could overcome the artifact and background enhancement from catheter and contrast in PA-MSCT. The absence of pulmonary arterial blood supply of the inoculated nodules may be due to the more central location and the big tumor size. Meanwhile, the deposition of lipiodol in two of the small distant metastases indicating pulmonary arterial blood supply may be well explained by the peripheral location and small size.

Instead of using suspension of tumor fragments (≤0.5 mm) for inoculation as in the report of Ahrar et al. (14), we have tried tissue blocks (1.5~2.0 mm) and observed a similar growth pattern. This simplified method may be considered in future experiment.

Several limitations of our study have to be acknowledged. First, the metastatic nature of this model, along with immunosuppression of the animal is an important limitation. But considering no ideal large animal model for lung cancer so far, the present model may be useful for experiments on transbronchial arterial intervention of lung cancer, at least for acute experiments such as pharmacokinetic studies. Second, blood supply was only evaluated at late stage, and pulmonary artery may supply or partly supply the tumors at their early growth. Besides, all the tumors were centrally located. Evaluation of the blood supply of tumors at different stages and at peripheral sites is needed. Third, no histological and recent CT perfusion analyses of blood supply were performed, although we believe that PA-MSCT and PA-L-MSCT were reliable to evaluate the pulmonary arterial supply. Finally, radiation exposure may be a concern because animals underwent multiple CT and DSA. Unfortunately, radiation dose at every single CT or DSA evaluation was not recorded, and it was not clear if the total dose had reached lethal dose. Now that the tumor growth and the blood supply have been revealed, CT and DSA evaluations may be reduced in future studies.

In conclusion, we demonstrated bronchial arterial blood supply of this CTVT lung tumor model, indicating that this model may be used for fundamental research on locoregional (both percutaneous and transarterial) treatment options for human lung cancer.

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Conflict of interest disclosure

The authors declared no conflicts of interest.

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