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Short Communication

Visualization of Tumor Vascularity in an Orthotopically Implanted Pulmonary Tumor Murine Model using X-ray Micro-Computed Tomography Imaging

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- VRT

Abstract

Objective: To visualize the vascularity of orthotopically implanted pulmonary tumor in a clinically relevant murine model of human Non-Small Cell Lung Cancer (NSCLC).

Method: Orthotopic pulmonary tumors in mice were established by intrapulmonary injection of human NSCLC Calu-6 cells. Contrast-enhanced micro-computed tomography (micro-CT) was used to visualize the pulmonary tumor and blood flow in the chest. To create the three-dimensional (3D) image, Digital Imaging and Communications in Medicine (DICOM) data from micro-CT acquisitions were transferred to surface-rendering software.

Result: The pulmonary tumor and vascularity in chest cavity were detected using micro-CT imaging with contrast agent. The 3D image created using surface-rendering software enabled clear visualization of the vascularity surrounding the pulmonary tumor and the blood vessels directly connected to the pulmonary vein.

Conclusions: The combination of contrast-enhanced micro-CT imaging and 3D imaging visualization using the volume rendering technique (VRT) was useful in evaluating the vascularity of pulmonary tumors in mice.

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ABBREVIATIONS

CT: Computed Tomography; NSCLC: Non-Small Cell Lung Cancer; DICOM: Digital Imaging and Communications in Medicine; 3D: Three-Dimensional; VRT: Volume Rendering Technique

INTRODUCTION

Tumor angiogenesis, the physiological process of *de novo* synthesis of blood vessels around a tumor, is required for tumor growth to larger tumors to obtain nutrients and oxygen, and waste product removal from the tumor [1]. Given that tumor cells in cancer patients can be spread to proximal and distal organs *via* metastasis through newly synthesized blood vessels [2], monitoring angiogenesis is one method of facilitating understanding of disease physiology. Lung cancer is a major concern with human health care, representing the leading cause of cancer-related deaths among both men and women worldwide [3]. Preclinical studies, including animal models and studies on methodology of disease monitoring, may aid in improving understanding of pathophysiological process and progression of the disease [4].

We previously reported that micro-computed tomography (micro-CT) imaging was useful in evaluating tumor progression in a murine model of orthotopoically implanted pulmonary tumor, in which a pulmonary tumor nodule had developed in the lung [4]. In the present study, we attempted to visualize the vascularity of a pulmonary tumor using contrast-enhanced x-ray micro-CT imaging with Volume Rendering Technique (VRT)-based three-dimensional (3D) imaging was critically useful to recognize the vascularity in the nature of 3D-architect. The vasculature of pulmonary tumor was developed at around the tumor burden, and 3D imaging revealed that the vasculature of pulmonary tumor in this model was directly connected to pulmonary vein. These results suggested that contrast-enhanced micro-CT imaging combined with surface-rendering 3D-image was useful to understand the angiogenesis of lung cancer in mice, and was helpful for drug evaluation of anti-angiogenic drugs in preclinical.

MATERIALS AND METHODS

Cancer cell line and mice model

Human Calu-6 cells purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) were routinely cultured and maintained in Minimal Essential Medium (MEM) + Gluta MAX medium (Invitrogen, Tokyo, Japan) supplemented with 10% Fetal Bovine Serum (FBS), 1 mM sodium pyruvate, and 17.9 mM sodium bicarbonate. Cells were passaged no more than six times. Male athymic mice (NAnN.Cg-Foxn1nu/CrlCrlj) were obtained from Charles River Laboratories Japan, Inc. (Yokohama, Japan). All mice were maintained and handled in accordance with the recommendations of the National Institute of Health (Bethesda, MD, USA). Intra-pulmonary implantation was previously described [4]. Briefly, nude mice (6 weeks old) were anesthetized with isoflurane (Forane; Abbott Japan, Tokyo, Japan), and a small skin incision was then made on the left chest wall. While monitoring the motion of the left lung, 20 μl of the cell suspension was injected directly into the lung using a 0.3-ml syringe equipped with a 29-gauge needle (Beckton & Dickinson, Tokyo, Japan). The skin incision was then closed with a surgical skin clip.

X-ray micro-CT and 3D analysis

X-ray micro-CT scans were conducted using an Inveon multimodality system (Siemens, Knoxville, TN, USA). CT scanning was performed under the condition of parameters followed as: 360 steps in 360°, matrix size: 2560 × 2048, Binning 2, 80 kV, 500 μA, exposure: 500 ms. Calu-6 tumor-bearing mice received an intravenous injection of iodine-based contrast agent (Iopamidol) at 500 μl. Digital imaging and communications in medicine (DICOM)-formatted data from CT acquisition were transferred to surface-rendering software (Real INTAGE; KGT, Tokyo, Japan). The pulmonary tumor burden was manually isolated with the view of CT image, and the vascular regions were detected based on the threshold of the AU signal.

RESULTS AND DISCUSSION

To visualize the vascularity of a pulmonary tumor, a mouse model of orthotopic human NSCLC was established. Newly synthesized blood vessels were maturated using contrast-enhanced X-ray micro-CT imaging performed at five weeks after implantation. As shown at Figure 1A, the implanted pulmonary tumor nodule was focally detected in the left lung lobe. Contrasted regions were observed in the heart, large blood vessels, and the shape of pulmonary tumor burden.

Spatial information of vascularity was obtained by generating a 3D image using surface-rendering software. A pulmonary tumor nodule was noted on the left side of heart (Figure 1B, C; shown as green). Tiny and fragile vascularity—possibly newly synthesized—was observed at the edge of the tumor and was directly connected to the pulmonary vein (Figure 1C). The pulmonary vein is an attractive target for vasculature attachment, as blood in this vessel contains the highest concentration of oxygen in the body, allowing the tumor nodule to obtain sufficient oxygen and nutrition for growth.

VRT analysis facilitates recognition of spatial locus as well as measurement of tumor vasculature volume. Savai et al. demonstrated the utility of micro-CT imaging with VRT in visualizing and quantitating tumor vascularity in LLC1 and A549 lung tumor models using an intra-tracheal implantation model [5]. In contrast to these previous lung cancer models, the intra-pulmonary injection approach in the present study produced a focal tumor nodule, in which neovascularization of pulmonary tumor might growth to be maturated and facilitated observation by micro-CT imaging.

CONCLUSION

Our findings here highlighted the utility of a combination of contrast-enhanced micro-CT imaging and VRT-based 3D-image analysis in evaluating the vascularity of lung tumor angiogenesis and anti-angiogenic drug effects in a mouse model of orthotopic lung cancer.

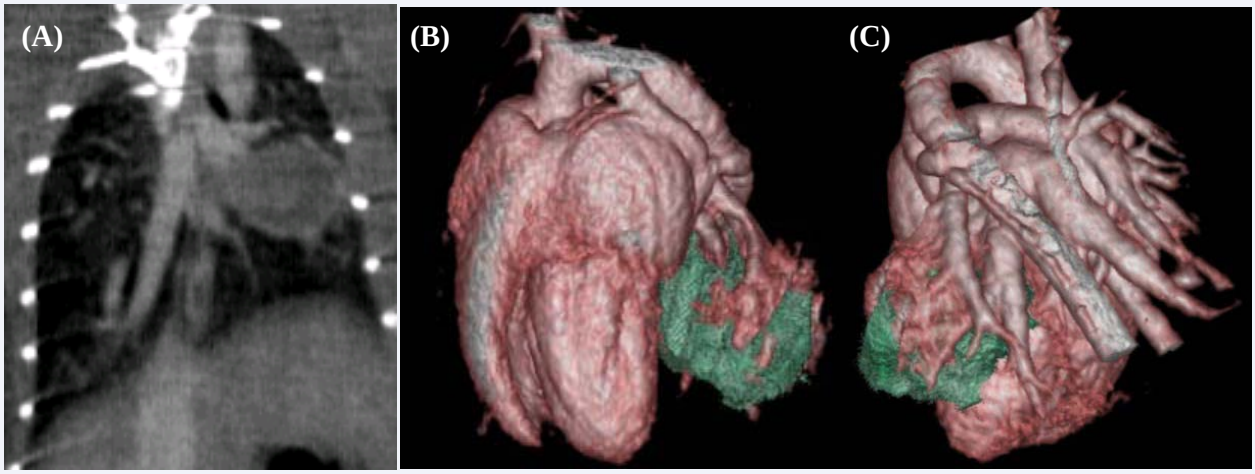

Figure 1 Contrast-enhanced micro-CT imaging of orthotopically implanted pulmonary tumor. Mice received an intra-pulmonary injection of Calu-6 (human NSCLC) cells (2×10^6 cells in 20 µl). Five weeks after transplantation, contrast-enhanced micro-CT acquisition was performed. (A) Slice image and (B) ventral and (C) dorsal views of 3D images reconstructed from DICOM data of X-ray micro-CT images are shown. Pink and green colors indicate vasculature and pulmonary tumor, respectively.

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Conflicts of Interest

All authors are employees of Astellas Pharma Inc.

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