Evaluation of functional and structural characteristics of tumor angiogenesis in lung cancer overexpressing different VEGF isoforms in a murine xenograft model by using of MR imaging

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Synopsis
Vascular endothelial growth factor (VEGF) is important for tumor associated angiogenesis. However, the different effects of VEGF isoforms in functional and structural characteristics of tumor angiogenesis is still unclear. The purpose of this study was to use dynamic contrast enhanced MRI and steady state contrast enhanced MRI to evaluate the in vivo vascular function using vascular transfer constant (Ktrans) and structural characteristics including microvessel density and vessel size of tumor angiogenesis induced by different VEGF isoform in non-small cell lung cancer.

Introduction
Angiogenesis is important for tumor growth and metastasis. VEGF (VEGF-A) is a well-known potent angiogenesis factor, and the alternative splicing of VEGF gene give rise to several isoforms [1]. The different effects of VEGF isoforms in functional and structural characteristics of tumor angiogenesis is still unclear. MRI is widely applied for functional and structural evaluation of tumor angiogenesis. Dynamic Contrast Enhanced MRI (DCE-MRI) using Tofts model, can provide a vascular functional parameter-vascular transfer constant (Ktrans) [2]. In addition, steady state contrast enhanced MR imaging (SSCE-MRI) with transverse relaxation rate shift (ΔR2 and ΔR2*) based on both spin echo (SE) and gradient echo (GE) imaging before and after injection of iron nanoparticle, may give structural information (relative vessel size imaging (rVSI) and relative vessel density imaging (rVDI)) on tumor angiogenesis [3-4]. The aims of this study were to use DCE- and SSCE-MRI to (1) evaluate whether the Ktrans of tumor angiogenesis were different among tumors overexpressing three different VEGF isoform (ie. VEGF121, VEGF165 or VEGF189), (2) evaluate the rVDI of tumors overexpressing three different VEGF isoform, and (3) investigate the rVSI in tumors overexpressing three different single VEGF isoform in a murine tumor xenograft model of human non-small cell lung cancer.

Material and Method
We established several clones of CL-1-0 lung cancer cell line (with low endogenous VEGF expression) with trasfection and overexpression of one of the three major VEGF isoforms, including VEGF121, VEGF165 and VEGF189. CL-1-0 lung cancer cell with overexpressing different VEGF isoforms and the mock clone were transplanted subcutaneously into a severe combined immunodeficient (SCID) mice. All MR experiments were performed on a horizontal 7.0 T Pharma Scan 70/16 spectrometer. The Ktrans and the rVDI and rVSI of the tumor microvessel were evaluated by DCE-MRI and SSCE-MRI. DCE-MRI was performed using a T1-weighted spin-echo sequence with a TR of 400 ms, TE of 10 ms, FOV of 3 cm, slice thickness of 1.5 mm, NEX of 1 and matrix size of 256x64 (zero filled to 512x256). A series of 40 axial imagings were acquired, i.e., before, during and after the orbital injection of Gd-DTPA (0.2 mmol/kg, Berlex, USA) after the 4th cycle. The structural properties of tumor vasculature were assessed by SSCE-MRI using a T2-weighted spin-echo sequence with a TR of 5000 ms, TE of 70 ms, FOV of 3 cm, slice thickness of 1.5 mm, NEX of 2 and matrix size of 256x128, and T2*-weighted gradient-echo sequence with a TR of 700 ms, TE of 10 ms, FOV of 3 cm, slice thickness of 1.5 mm, NEX of 2 and matrix size of 256x128. T2-weighted spin echo and T2*-weighted gradient echo sequence will be employed and performed before and after the injection of iron oxide (Resovist) with dose of 30mg Fe/kg. The post-contrast imaging acquisition will be delayed by 5 minutes for ensuring a steady state distribution of contrast agent in the vascular network.

Data analysis
The kinetic analysis of dynamic Gd-DTPA signal enhancement was based on the Tofts model. In this analysis, the rate of contrast agent uptake, dCt/dt, can be determined by Ktrans, the tracer concentration in arterial blood plasma (Cp), the tracer concentration in tissue (Ct) and the leakage space per unit volume of tissue (ve), according to the [equation 1]: dCt/dt=K trans Cp(1-Cs)/ve Ct, where Cs, Cp, and the Ktrans are the pre-contrast and post-contrast signal intensities for spin echo and gradient echo, respectively. rVDI and rVSI will be derived from the ratio of relaxation rate shift, ΔR2/ΔR2* and ΔR2/ΔR2, respectively.

Results and Discussion
As shown in Fig.1, the Ktrans map on DCE-MRI showed that the perfusion and permeability signals was high and distributed from rim to core of VEGF 189 overexpressing tumor, which was in contrast to VEGF121 overexpressing tumor, which had low Ktrans signal located mainly in the rim of tumor. The Ktrans value was significantly higher in VEGF189 overexpressing tumor, both in rim and core region, than other VEGF isoform overexpressing and the mock tumor (p<0.05). The rVDI on SSCE-MRI showed that microvessel in tumor was most dense in VEGF 189 overexpressing tumor, and least dense in VEGF121 overexpressing tumor (p<0.05) (Fig. 2). The rVSI on SSCE-MRI showed that the size of microvessels was largest in VEGF121 overexpressing tumor, intermediate in VEGF165 overexpressing tumor and smallest in VEGF189 overexpressing tumor (p<0.05). Our data suggest that the VEGF189 overexpressing tumor had very dense and small size microvessel distributed from peripheral to center of tumor, and VEGF165 overexpressing tumor had dense and middle size microvessel located mainly in the peripheral part of tumor, and VEGF121 overexpressing tumor had least dense and large size microvessels in the peripheral of the tumor.

Conclusions
We concluded that among three major VEGF isoforms, VEGF189 isoform can induce most dense and smallest sized sprouting tumor microvessel that penetrated from peripheral to center of tumor, and had highest in vivo Ktrans index in non-small cell lung cancer. This may contribute to the adverse effect of VEGF189 overexpressing on patient’s prognosis in several human cancers including non-small cell lung cancer.

References

Fig.1. In vivo Ktrans and values of CL-1-0 cancer cells overexpressing VEGF isoforms

Fig.2. In vivo relative vessel density (rVDI) and size (rVSI) map and values of CL-1-0 cancer cells overexpressing VEGF isoforms