Simultaneous determination of blood flow, microvascular permeability and blood volume in lung cancer overexpressing different VEGF isoforms in a murine xenograft model by dynamic contrast enhancement MR imaging (DCE-MRI)

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Synopsis
Vascular endothelial growth factor (VEGF) is a key mediator of tumor angiogenesis. However, biologic effects of different VEGF isoforms (such as VEGF121, VEGF189 and VEGF206) on in vivo tumor associated angiogenesis is still not clear. Adiabatic approximation to the tissue homogeneity model (AATH model) is a newly developed which can be used to simultaneously produce specific angiogenic parameters, including blood flow (Fp), permeability (PSp) and blood volume (Vb). In the present study, we apply AATH model to assess angiogenesis in lung cancer overexpressing different VEGF isoforms in a murine xenograft model.

Introduction
Development of angiogenesis in a tumor will lead to an increase in blood flow (Fp), permeability (PSp) and blood volume (Vb). Hence, it is important to use Fp, PSp and Vb as indices to evaluate tumor angiogenesis. However, conventional DCE-MRI using T1 T2, modes can only measure combination of Fp and PSp in Ktrans under the effect of Vb, but not the individual quantities. Recently, the adiabatic approximation to the tissue homogeneity model (AATH model) [1] can separate Fp and PSp in transfer constant (Ktrans) and obtain Vb. It allows simultaneous measure of tumor microvasculature parameters of Fp, PSp and Vb. Vascular endothelial growth factor (VEGF) is a key mediator to tumor associated angiogenesis [2]. The biologic effects of different VEGF isoforms (such as VEGF189, VEGF206 and VEGF165) on in vivo tumor associated angiogenesis is still not clear. Our aim is to apply AATH model coupled with the direct measurement of arterial input function (AIF) obtained from the left ventricle (LV) of heart to simultaneously assess Fp, PSp and Vb in lung cancer overexpressing different VEGF isoforms in a murine xenograft model.

Material and Method
We constructed different VEGF isoform cDNA in pTR2 vectors, and transfected these vectors into a non-small-cell lung cancer (NSCLC) cell line with minimal native VEGF production (CL1-O). The VEGF isoform overexpressing cells were subcutaneously implanted on 12 male SCID mice. All MR experiments were performed on a horizontal 7.0 T Pharma Scan 70/16 spectrometer. For the determination of AIF, we imaged tumor and left ventricle blood pool simultaneously by T1-weighted FLASH sequence with a TR of 10.7ms, TE of 2.2 ms, FOV of 4cm, slice thickness of 2mm, NEX of 1, matrix size of 256x128 and Flip angle of 90°. The sequence was triggered by the R-wave on the electrocardiogram (ECG). DCE-MRI was performed using a T1-weighted FLASH sequence with a TR of 30ms, TE of 1.8 ms, FOV of 4cm, slice thickness of 2mm, NEX of 1, matrix size of 128x64 and Flip angle of 90°. A series of 120 axial imagings were acquired, i.e., before, during and after the i.v. injection orbital of Gd-DTPA (0.1mmol/kg, Berlex, USA) after the 30th cycle.

Data Analysis
The kinetic analysis of dynamic Gd-DTPA signal enhancement was based on the adiabatic approximation to the tissue homogeneity model (AATH model). AATH model predicts how the time course of contrast agent in tumor (the tissue residue function, Ct) is dependent on the vascular parameters (Fp and PSp) and the time course of contrast agent in an artery (the arterial input function, Ca): Where

\[ C(t) = \frac{Fp}{C(a)} \frac{1}{R(t)} \]  
\[ R(t) = 0 \quad 0 \leq t \leq Tc \]

\[ \text{denotes the impulse residue function.} \]
\[ Tc \text{ (seconds) is the mean capillary transit time.} \]
\[ \text{Ve (unitless) is the volume of the interstitial space, and} \]
\[ E \text{ is extraction ratio.} \]
\[ Fp, E, Tc \text{ was obtained from numerical deconvolution.} \]
\[ \text{PSp was calculated from Fp and E, so we can obtain PSp = Fp \times (1-E).} \]
\[ \text{Vb (blood volume) was determined from the product of Fp and Tc/60 [1].} \]
\[ \text{The processing software for quantitative analysis of the dynamic curve was written in Matlab (Math Works, Natick, MA, USA).} \]
\[ \text{The ROIs were selected from Fp, PSp and Vb map and their corresponding T2WI.} \]
\[ \text{Tumor core was chosen from the inner 50% of the tumor area and rim was chosen from the outer part of the tumor area.} \]

Results and discussion
A typical cardiac short axis imaging of a mouse bearing a tumor on its back is shown in Fig. 1a. The left ventricle (LV), right ventricle (RV), and tumor tissue are well visualized in the short axis as arrow indicated. A typical AIF following bolus contrast agent injection is depicted in Fig1b, while the tumor area and rim was chosen from the outer part of the tumor area.

Table 1. Transfer constant, blood flow, permeability, and blood volume for different VEGF isoforms overexpressing tumors in tumor rim / core

<table>
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<th>VEGF Isoform</th>
<th>Blood Flow (ml/min/g)</th>
<th>Microvascular Permeability (PSp)</th>
<th>Blood Volume (ml/g)</th>
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<td>VEGF121</td>
<td>42.1 ± 19.4</td>
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<td>61.5 ± 6.5</td>
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<tr>
<td>VEGF189</td>
<td>41.8 ± 12.0</td>
<td>32.4 ± 10.5</td>
<td>60.4 ± 5.5</td>
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<tr>
<td>VEGF165</td>
<td>40.5 ± 11.2</td>
<td>29.8 ± 9.5</td>
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Table 2. Transfer constant, blood flow, permeability, and blood volume for different VEGF isoforms overexpressing tumors in tumor rim / core

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References

Fig. 1. (a) T1-weighted imaging of tumor and LV at the same slice, (b) AIF and (c) tumor concentration – time curves. (a-c) are representative results from one mouse.

Fig. 2. In vivo transfer constant (Ktrans), blood flow (Fp), microvascular permeability (PSp), and blood volume (Vb) of CL1-O cancer overexpressing VEGF isoforms.