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 $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$) on *in vivo* tumor associated angiogenesis is still not clear. Adiabatic approximation to the tissue homogeneity model (AATH model) is a newly development which can be used to produce simultaneously 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 Fρ, PSρ and Vb as indices to evaluate tumor angiogenesis. However, conventional DCE-MRI using Tofts modes can only measure combination of Fρ and PSρ in K^{trans} under the effect of Vb, but not the individual quantities. Recently, the adiabatic approximation to the tissue homogeneity model (AATH model) [1] can separate Fρ and PSρ in transfer constant (K^{trans}) and obtain Vb. It allows simultaneous measure of tumor microvasculature parameters of Fρ, PSρ and Vb. Vascular endothelial growth factor (VEGF) is a key mediator for tumor associated angiogenesis [2]. The biologic effects of different VEGF isoforms (such as $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$) 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 Fρ, PSρ 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 (CL1-0). 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 30^0. 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 90^0. A series of 120 axial imaginges 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):

$$C_t(t) = F\rho C_a(t) \otimes R(t) \qquad R(t) = \begin{cases} 0 & 0 \le t \le Tc \\ Ee^{-\frac{EF}{Ve}(t-Tc)} & t \ge Tc \end{cases}$$

Where $\otimes$ denotes the impulse residue function. Tc (seconds) is the mean capillary transit time, Ve (unitless) is the volume of the interstitial space, and E is extraction ratio. Fp, E, Tc was obtained from numerical deconvolution**.** PSp was calculated from Fp and E, so we can obtain PSp= -Fpln(1-E), Vb (blood volume) was determined from the product of Fp and Tc/60 [1]. The processing software for quantitative analysis of the dynamic curve was written in Matlab (Math Works, Natick, MA, USA). The ROIs were selected from Fρ, PSρ and Vb map and their corresponding T2WI. 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 concentration-time curve, showing contrast agent washin and washout in tumor, is shown in Fig1c. After deconvolution of AIF and tumor concentration-time curves, and curve fitting pixel by pixel, the different functional maps of tumor angiogenesis were obtained as shown in Fig.2. Quantitative analyses of functional maps show that the K^{trans}, Fρ, PSρ, and Vb values are higher in VEGF 189- overexpressing tumors than VEGF 121- and VEGF165-isoform overexpressing tumors and mock in tumor rim and core ($p<0.05$) as illustrated in Table 1. The results showed that the higher K^{trans} values in VEGF 189 overexpressing tumor are due to increases in both blood flow and permeability. This suggested the VEGF189 overexpressing tumor can obviously up-regulated angiogenesis which are associated with higher sufficiency in tumor's blood supply (blood flow and volume)and more leakage of protein (permeability) in blood vessels, and this may facilitate tumor growth, and metastasis of lung cancer.

Conclusion

In the present study, we have shown that (1) MRI with AATH model using Fρ,PSρ and Vb parameter can assess the blood flow, permeability and blood volume individually of tumor angiogenesis in non-small cell lung cancer. (2)The Fp, PSp and Vb showed that the blood flow, permeability and blood volume is highest in VEGF 189 overexpressing tumor as compared to other VEGF isoforms overexpressing tumors. The high blood flow, vascular permeability and blood volume in VEGF 189 inducing angiogenesis may contribute to more aggressive biological behaviors such as rapid tumor growth and early metastasis of tumor which overexpressing VEGF 189 isoform in NSCLCs.

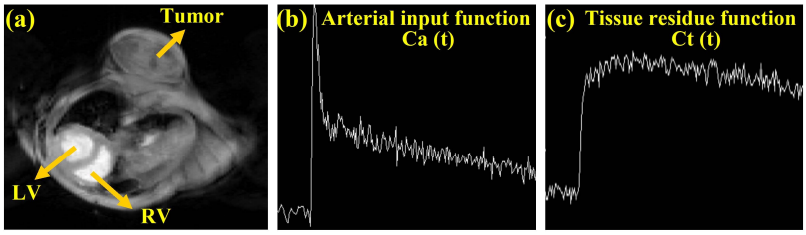

Fig.1. (a) T1-weighted imaging of tumor and LV at the same slice, (b) AIF and (c) tumor concentration – time curves. (a-c) are representative results from one mouse

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

	Different VEGF isoform overexpressing tumors			
Perfusion Measurement	**Mock**	**VEGF121**	**VEGF165**	**VEGF189**
Transfer Constant (1/min)	0.09±0.05 / 0.08±0.03	0.19±0.03 / 0.03±0.02	0.31±0.05 / 0.07±0.01	0.6±0.13 / 0.4±0.11
Blood Flow (ml/100g/min)	42.1±19.4 / 35.5±17.6	61.5±6.5 / 15.6±6.4	82.8±13.4 / 32.4±6	131.6±12 / 100.2±17
Permeability (ml/100g/min)	15.5±8.4 / 12.8±5.4	36.6±6 / 4.4±2.7	67.2±12.3 / 11.9±2.3	147.4±42.5 / 89±29.3
Blood Volume (ml/100g)	6.1±4 / 5.4±3.7	8.1±0.6 / 2.4±0.5	9.3±2.1 / 5.8±0.8	14.9±0.6 / 9.9±0.9

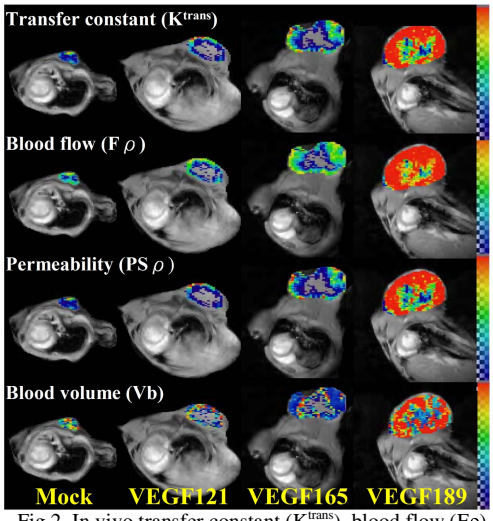

Fig.2. In vivo transfer constant (K^{trans}), blood flow (Fρ), permeability (PSρ), and blood volume (Vb) of CL1-0 cancer overexpressing VEGF isoforms

References

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