Evaluation of functional and structural characteristics of tumor angiogenesis in lung cancer overexpressing different VEGF isoform 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 (such as $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$) 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 (K^{trans}), 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 (K^{trans}) [2]. In addition, steady state contrast enhanced MR imaging (SSCE-MRI) with transverse relaxation rate shift ($\Delta R2$ and$\Delta R2^*$) 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, $\Delta R2^*/\Delta R2$) and relative vessel density imaging (rVDI, $\Delta R2/\Delta R2^*)^{2/3}$) on tumor angiogenesis [3~4] . The aims of this study were to use DCE- and SSCE- MRI to (1) evaluate whether the K^{trans} 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 CL1-0 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. CL1-0 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 K^{trans} 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 400ms, TE of 10 ms, FOV of 3 cm, slice thickness of 1.5mm, NEX of 1 and matrix size of 256x 64 (zero filled to 256×256). A series of 40 axial imaginges 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 1.5mm, NEX of 2and matrix size of 256x 128, and $T2^*$-weighted gradient-echo sequence with a TR of 700ms, TE of 10 ms, FOV of 3 cm, slice thickness of 1.5 mm, NEX of 2 and matrix size of 256x 128. 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 K^{trans}, 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]: $dCt(t)/dt=K^{trans}\,Cp(t)-(K^{trans}/v\,e)\,Ct(t)$. The processing software for quantitative analysis of the dynamic curve was written in Matlab (Math Works, Natick, MA, USA) .The ROIs were selected from K^{trans} 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. Transverse relaxation rate change will be given by $\Delta R_2=\ln(S_{pre}/S_{post})/TE$ and $\Delta R_2^* = \ln(S_{pre}^*/S_{post}^*)/TE$, where S_{pre}, Spost, S_{pre}^* and S_{post}^* 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, $\Delta R2/(\Delta R2^*)^{2/3}$ and$\Delta R2^*/R2$, respectively.

Results and Discussion

As shown in Fig.1, the K^{trans} 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 K^{trans} signal located mainly in the rim of tumors. VEGF165 overexpressing tumor had low to middle K^{trans} signal that distributed mainly in the rim of tumor. The K^{trans} value was significantly higher in VEGF 189 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 VEGF189 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, 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* K^{trans} 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

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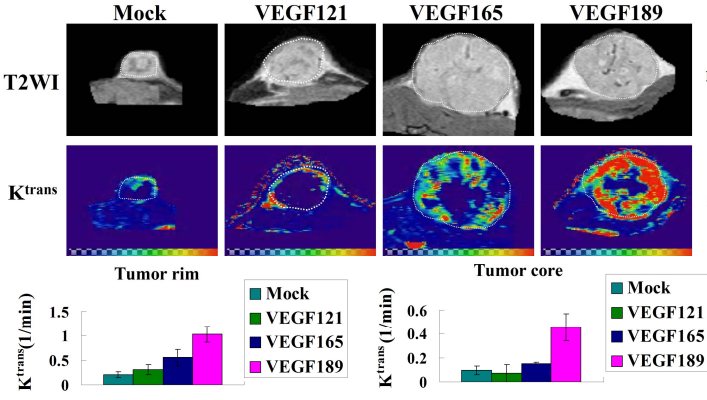

Fig.1. In vivo K^{trans} and values of CL1-0 cancer cells overexpressing VEGF isoforms

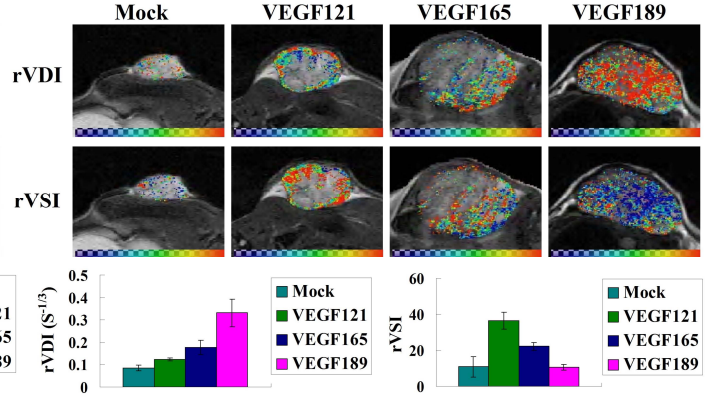

Fig.2. In vivo relative vessel density (rVDI) and size (rVSI) map and values of CL1-0 cancer cells overexpressing VEGF isoforms