Evaluation of tumor angiogenesis and growth rate in lung cancer overexpressing four different VEGF isoforms in a murine xenograft model

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Synopsis

Vascular endothelial growth factor (VEGF) is a key mediator of tumor angiogenesis. In the present study, dynamic contrast enhanced magnetic resonance imaging was used to evaluate the relationship between angiogenesis and tumor growth in non-small cell lung cancer overexpressing four different VEGF isoforms in a murine xenograft model. The results demonstrate that different VEGF- overexpressing tumors exhibit different angiogenesis activity leading to different tumor growth rates.

Introduction

The necessity of angiogenesis in tumor growth is well understood. Accumulating evidences suggest that the onset of angiogenesis can occur at any stage of tumor progression. Vascular endothelial growth factor (VEGF) is important for tumor associated angiogenesis. Four different isoforms of VEGF-A family (VEGF121, VEGF165, VEGF189, and VEGF206) have been reported to possess different biological activities [1]. However, the biologic effects of different VEGF isoforms on *in vivo* tumor associated angiogenesis are still not clear. The purpose of the present study is to investigate the correlation between angiogenesis and tumor growth in lung cancer overexpressing the four aforementioned VEGF isoforms in a murine xenograft model using dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) [2].

Material and Method

Four different VEGF isoform cDNA in pTR2 vectors were constructed, and then transfected into a non-small-cell lung cancer cell lines with minimal native VEGF production (CL1-0). The anesthetization of animals were induced with 4 % isoflurane in O_2 (4 L/min) and maintained at 1.5 %. The body temperature was kept with warm water circulation at 37±1°C. All MR experiments were performed on a horizontal 7.0 T Pharma Scan 70/16 spectrometer equipped with an active shielding gradient (30 G/cm in 150 μ s) operating on a paravision (version 3.0.1) software platform. A 38mm birdcage coil was used for RF excitation and signal reception, and no triggering was used for data acquisition. Experiments were started from the 7th day after the implantation of the tumor and were carried out at specific intervals (Days 7, 14, 17, 21, 28, 35).The total tumor volume was obtained from the multi-slice axial T2-weighted images: TR of 5000 ms, TE of 10 ms, FOV of 3 cm, NEX of 2, matrix size of 256x256, and slice thickness 0.5 mm with no interslice gap to cover the entire tumor. On each tumor slice, ROI was manually outlined, and the total tumor volume was calculated as the sum of all ROIs from each individual slice**.** 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. A series of 40 axial imaginges were acquired, i.e., before, during and after the orbital injection of contrast agent gadopentic acid (Gd-DTPA, 0.2 mmol/ kg, Berlex, USA) after the 4th cycle.

Data analysis

The tumor growth rate was quantified as volumetric doubling time (Td), based on the classic model of tumor growth, V(t) = V(0)*2t/Td [equation 1], where V(t) is total volume at time t, Td is the volumetric doubling time and V(0) is the initial tumor volume. The kinetic analysis of dynamic Gd -DTPA signal enhancement was based on the compartment model of Tofts and Kermode. In this analysis, the rate of contrast agent uptake, dCt/dt, can be determined by transform constant (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 2]: $dCt(t)/dt=K^{trans}\,Cp(t)-(K^{trans}/v\,e)\,Ct(t)$. 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.

Results and Discussion

T2WI were used to determine the volumes of individual tumors from day7 to day35 (Fig1a). At day35, the size of VEGF165- and VEGF189- overexpressing tumor (647.7 mm^3, 371.1 mm^3, respectively) was significantly larger than VEGF121-, VEGF206- overexpressing tumor, and mock. (98.2 mm^3, 11.37 mm^3 and 37.9 mm^3, respectively) ($p<0.01$) (Fig1b). Fig1c show smaller doubling time (Td, as defined in [equation1]) of VEGF 165- and 189- overexpressing tumor than others ($p<0.01$), suggesting fast tumor growth in VEGF 165- and 189- overexpressing tumor. Our results suggested that VEGF165- and VEGF189- overexpressing tumor result in higher cell proliferation in tumor. Fig2a displayed color K^{trans} map obtained by curve fitting [as defined in equation2] of CL1-0 cancer cells overexpressing four VEGF isoforms at day14 and day35. The measurement of K^{trans} was performed on the tumor rim (Fig2b) and tumor core (Fig2c) in four different isoform overexpressing tumors. At day 14, there were essentially no different K^{trans} values were observed between tumor rim and core. By contrast, at day35, K^{trans} values are higher in VEGF 189 overexpressing tumors than mock ,VEGF 121- ,VEGF165- and VEGF206- isoform overexpressing tumors in tumor rim and tumor core ($p<0.05$). The result suggested that high growth rate in VEGF189 overexpressing tumors may be the consequence of higher angiogenesis.

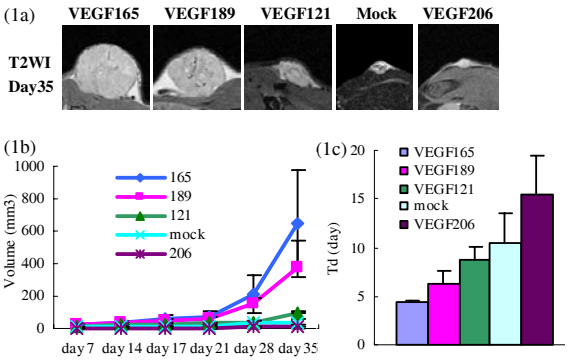

Fig.1 Representative T2WIs (a), the tumor growth curves (b), and the tumor doubling time (c) from day7 to day35 in in vivo tumorigensis of

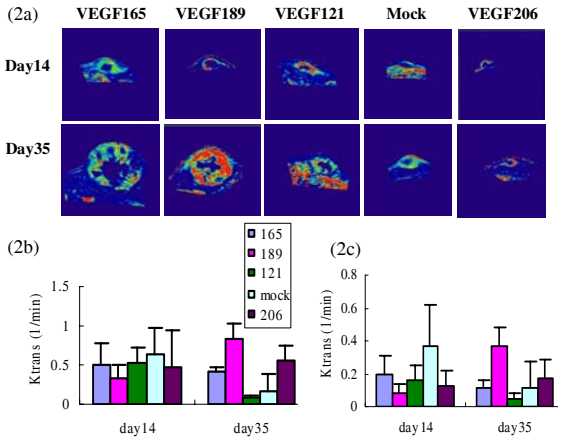

Fig.2 Representative the K^{trans} color maps (a), K^{trans} values in tumor rim (b) , and K^{trans} values in tumor core (c) of CL1-0 cancer cells overexpressing four VEGF isoforms

Conclusions

In the present study, we have shown that (1) DCE-MRI can be used to evaluate the function of tumor angiogenesis *in vivo* in non-small cell lung cancer overexpressing different VEGF isoforms and (2) the angiogenesis activity and tumor microvessel function is higher in VEGF 189 overexpressing tumor than mock and other VEGF isoform overexpressing tumors, this partially explains the higher tumorigenesis in VEGF 189 overexpressing non-small cell lung cancers.

References

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