# VISUALIZATION OF TUMOR ANGIOGENESIS IN LUNG CANCER OVEREXPRESSING DIFFERENT VEGF ISOFORM IN A MURINE XENOGRAFT MODEL BY USING HIGH RESOLUTION 3DIMENTIONAL CONTRAST-ENHANCED MICROSCOPIC MR ANGIOGRAPHY

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**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 gives rise to several isoforms, such as $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$ [1]. It can provide an important new insight into the process of tumor angiogenesis to evaluate the effects of different VEGF isoforms on tumor feeding vessels and intratumor vessels in non-small cell lung cancer. Currently, there are several MR Angiography (MRA) methods including time-of –flight, phase contrast, and contrast-enhanced MRA, which have been employed to direct visualize vascular network. Among them, contrast-enhanced MRA based injection of contrast agent to shorten T1 relaxation time is the most sensitive for assessing tumor feeding vessels and intratumor vessels [2]. Therefore, the aim of this study was to evaluate and to visualize tumor feeding vessels and intratumor vessels in lung cancer overexpressing different VEGF isoform in a murine xenograft model by using High Resolution 3Dimentional Contrast Enhanced- Microscopic MR Angiography (HR 3D CE-mMRA).

**Material and Method**

The CL1-0 lung cancer cells were transfected and overexpressed different VEGF isoforms including VEGF121, VEGF165 and VEGF189. Those transfected cells and mock clone were then transplanted subcutaneously into a severe combined immunodeficient (SCID) mouse. All MR angiography were performed on a horizontal 7.0 T Pharma Scan 70/16 spectrometer. T1weighted imaging was acquired by using 3D fast low angle shot sequence after injection of contrast agent (Gd-DTPA, Berlex, USA, 0.1mmol /kg) with a TR of 20 ms, TE of 3 ms, FOV =6.2 cm ×3cm × 3 cm, acquisition matrix = 256×128×128 (zero-padded to 512×256×256). The resolution was 121×117×117μm. Microvasculature image was constructed with HR 3D CE-mMRA by using maximum intensity projections. Signal to noise ratio (SNR) was calculated as Signal/Noise [3]. ROI was placed on 2D CE-mMRA which has largest tumor section from 3D CE-mMRA. Tumor core was chosen from the inner part which was 50% of the tumor area; rim was chosen from the outer part which was 50 % of the tumor area.

**Results and discussion**

HR 3D CE-mMRA provided a complete view of tumor vessel network and revealed some feeding blood vessels growing into tumor overexpressing different isoform of VEGF. In the mock tumor, the result showed a few feeding vessels surrounding the tumor, and only a few scattered vessels in the tumor rim (Fig.1a). In the VEGF121-transfected tumor, some feeding vessels were observed outside the tumor, and vessel signals increased in the rim (Fig.1b). In the VEGF165- overexpressing tumor, the result showed increased number of feeding vessels outside the tumor (Fig.1c). More vessel signals were observed in the tumor rim and the tumor core. In the VEGF189-overexpressing tumor, a few dilation feeding blood vessels grew into tumor, and much more signals were distributed in the tumor rim and the tumor core (Fig1d). Among the different isoforms, VEGF189-overexpressed tumor had the highest SNR value in the tumor rim and the tumor core (Fig.2a, b). The vessel signals had lowest SNR in the VEGF121- overexpresed tumor. The highest SNR in VEGF189-tumor indicated strong angiogenesis activity. The higher angiogenesis and dilation of feeding vessel with VEGF189 helped transport nutrients and oxygen into the tumor. The dilation of feeding vessels resulted in a reduced resistance of vessels and an increased blood flow [2]. These results contributed aggressive biological behaviors of cancer cell, such as rapid tumor growth and early metastasis in non-small cell lung cancer.

**Conclusion**

In the present study, we have shown that HR 3D CE- mMRA can provide a complete view of the entire tumor vasculature in 3 dimension, especially the feeding vessel and intratumor vessel in tumor. This method can be a potential tool to evaluate *in vivo* angiogenesis phenotype induced by the different isoform of VEGF in non-small cell lung cancer.

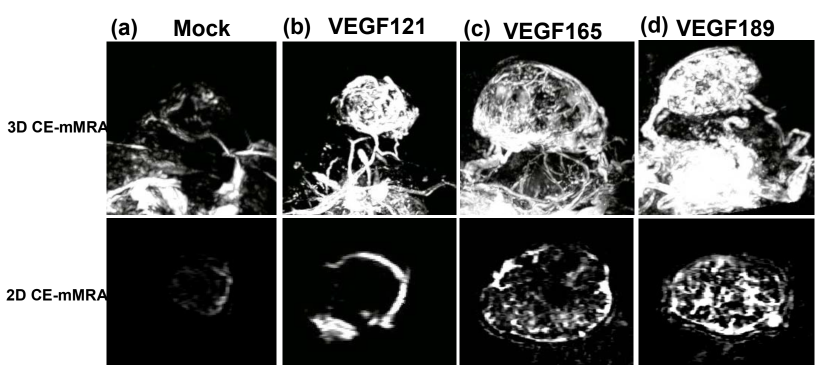


Fig.1 In vivo (a) Mock (b) VEGF121 (C) VEGF165 (D) VEGF 189 overexpresed tumor in HR 3D/ 2D CE-mMRA

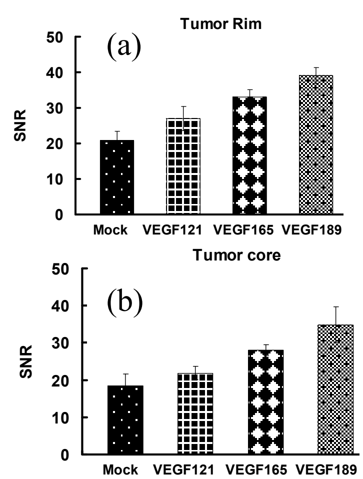


Fig.2 In vivo SNR value in the rim (a) and core (b) of CL1-0 cancer cells overexpressing VEGF isoform

**References**

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