Osteopontin regulates the functional responses to gas challenge of tumor angiogenesis
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Introduction
Angiogenesis, one of the cancer hallmarks, is critical in driving tumor progression and metastasis. Osteopontin (OPN), a member of the small integrin-binding ligand N-linked glycoproteins (SIBLINGs) family, is frequently overexpressed in glioblastoma and its expression is correlated with disease progression. To exploit the role of OPN in tumor progression and malignancy, the major goal of the present study is to elucidate the involvement of OPN in tumor angiogenesis, in particular its effect on both the functional and structural characteristics of the tumor vasculature. We studied the role of OPN in tumor angiogenesis in a xenograft glioma model using the rat C6 cells from which OPN expression was knocked down, along with the control knockdown (KD) cells. The functional and structural aspects of the tumor vasculatures were investigated by blood oxygenation level-dependent (BOLD) MRI with gas challenge and steady-state contrast-enhanced (SSCE) MRI, respectively. Our study provides direct in vivo evidence supporting a role of OPN in the functionality of tumor vasculature.

Material and Method
The rat C6 cells with OPN-KD or luciferase KD (Luc-KD, serving as control) were established by Lentivirus-based shRNA technology. A volume of 0.6 μL of the cell suspension (1x10⁶/10 μL in phosphate-buffered saline) was injected into 9-week-old Sprague–Dawley rats on the stereotactic apparatus using a 30-gauge needle (Hamilton, NV, USA) and a micro-infusion pump (Model 310; KD Scientific, MA, USA). The injection was made at the left striatum [Bregma=0.2mm, lateral=3.0mm, and depth=5.0 mm]. After recovery from surgery, the rats were scanned by a 7-T MRI system. For scanning, rats were anesthetized with isoflurane flowed in air (isoflurane at 5% for induction and 2% for maintenance), fitted in a custom designed head holder, and inserted into the magnet. T2-weighted images (T2WIs) were acquired at 24 days after C6 cells implantation using a fast spin echo sequence with field of view (FOV) =2.56 cm, slice thickness=0.5 mm, 8 slices with no gap to cover the whole tumor, TR=4000 ms, TE=70 ms, echo train length=8, number of excitations (NEX) =12, and matrix size=256x128 (zero filled to 256x256). To determine ∆R2* of gas challenge, T2*-weighted images (T2*WI) were performed while air and carbogen (5% CO₂/95% O₂) inhaling. Acquisition was delayed by 15 minutes after gas transition. T2*WI was acquired using 2D fast low angle shot (FLASH) sequence with a TR=200 ms, a TE=25 ms, flip angle=15°, voxel size=256×128 (zero filled to 256×256). To evaluate the role of OPN in tumor angiogenesis, in particular its effect on tumor angiogenesis, a xenograft glioma model using the rat C6 cells from which OPN expression was knocked down, along with the control knockdown (KD) cells. The functional and structural aspects of the tumor vasculatures were investigated by blood oxygenation level-dependent (BOLD) MRI with gas challenge and steady-state contrast-enhanced (SSCE) MRI, respectively.

Results and Discussion
Fig. 1A shows the T2WI, T2*WI with air, T2*WI with carbogen, and the ∆R2* maps. T2WI showed that the tumor sizes were invariable, but the T2*WI and ∆R2* maps demonstrate that the OPN-KD tumor showed higher reactivity to the gas challenge. The ∆R2* quantification was summarized in Fig. 1B. The ∆R2* signals of OPN-KD tumors were significantly higher than those in Luc-KD tumors (p<0.05). The hyperintensities on the ∆R2* maps may indicate the locations of functional vessels with oxygenation ability. According to the results, OPN-KD tumor exhibited significantly more functional intratumor vessels than the control tumor. In contrast, no unequivocal difference was identified in the structural features between OPN-KD and Luc-KD tumor vasculature such as vessel size and density (Images shown as Fig. 2A; Quantification summarized in Fig. 2B). In conclusion, the knockdown of OPN affects the functionality of tumor angiogenesis but preserves the structural features of tumor vasculature. The particular involvement of OPN in tumor angiogenesis provides an insight to the development of anti-tumor treatment.

Reference: J. Matusan-Ilijas K et al., 2008. 2. Saitoh Y et al., 1995.