Characterization of SCUBE3 protein for its role in tumor vascularization by SSCE-MRI

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Introduction

SCUBE3 (signal peptide-CUB-EGF-like domain-containing protein 3), firstly identified from human umbilical endothelial cells, is a secreted glycoprotein (1). The *SCUBE3* gene encodes for a polypeptide of 993 amino acids and the protein domain structure is composed of at least five motifs: an N-terminal signal peptide sequence, nine copies of EGF (epidermal growth factor)–like repeats, a spacer region followed by three repeated cysteine-rich domains and one CUB (complement proteins C1r/C1s, Uegf, and Bmp1) domain at C-terminus. SCUBE3 is not only expressed in vascular endothelial cells but also in osteoblasts and cardiac tissue. It has been reported that transgenic over-expression of SCUBE3 in the heart could stabilize the TGF-β1 signaling through releasing the EFG-like repeats from the CUB domain, and lead to cardiac hypertrophic response in murine model (2).Our previous study showed that SCUBE3 mRNA was up-regulated in highly invasive lung cancer cell lines and in patients with poor prognosis. These results prompt us to pursue the biological functions of SCUBE3 in lung cancer progression. Here we characterized the relevance of SCUBE3 expression and tumor proliferation, invasion, and angiogenesis on mouse model by using steady state contrast-enhanced-MRI method to cross-validate the in vitro study results.

Materials and Methods

Control stable cell line CL1-5 (shLacZ) and SCUBE3 knockdown stable cell line CL1-5 (shSCUBE3) were gifts from Dr. Pan-Chyr Yang. For tumor implantation, these cells were cultured in RPMI medium containing 10% FBS and puromycin (0.75 ug/ml) at 37°C in a humidified atmosphere of 5% CO_2-95% air. 5×10^6 cells were subcutaneously injected into 8-week-old male non-obese diabetic-severe combined immunodeficiency (NOD-SCID) mice. Tumor size was measured twice a week by ultrasound imaging using the formula: $V = ab^2/2$ where a is the length and b is the width of the tumor (3). Animal experiments were performed with the protocols approved by the Academia Sinica Institutional Animal Care and Utilization Committee.

The invasiveness of control stable cell line CL1-5 (shLacZ) and SCUBE3 knockdown stable cell line CL1-5 (shSCUBE3) was measured by detecting the circulating human tumor cells in the mouse model. The amount of circulating tumor cells in the mouse circulation system was measured using human *Alu* sequence as the marker (4).

All MR images were performed with a horizontal 7.0 T Pharma Scan 70/16 spectrometer (FMIC Academia Sinca) with a 38-mm volume coil as both the transmitter and receiver coil. On day 26 after tumor implantation, T2 weighted images (T2WI) and T2* weighted images (T2*WI) were obtained by SSCE-MRI before and after orbital vein injection with superparamagnetic iron oxide (SPIO, Resovist, schering). T2WI was obtained with fast spin echo sequence with TR of 5000 ms, a pseudoecho time of 70 ms, an echo train length of 8, FOV of 3 cm × 3 cm, acquisition matrix of 256x128 (zero-padded to 256×256); T2*WI was obtained with fast low-angle shot(FLASH) sequence of TR of 700 ms, TE of 10 ms, flip angle of 15, FOV of 3cm×3cm, acquisition matrix of 256x128 (zero-padded to 256×256). The signal intensities of precontrast (Spre, Spre*) and postcontrast (Spost, Spost*) were derived from images of T2WI, T2*WI, respectively. Transverse relaxation rates were then calculated by $\triangle R2$ (=ln (Spre /Spost)/TE) and $\triangle R2^*$ (= ln (Spre*/Spost*)/TE). VSI and VDI can be estimated by the following equation: $rVSI=(\triangle R2^*/\triangle R2)$ and $rVDI=\triangle R2/\triangle(R2^*)^{2/3}$. The processing software for the quantitative analyzes was written in MATLAB (MathWorks, Natick, MA, USA). In order to compare the vessel distribution at tumor core and rim, the boundary between rim and core of tumor was arbitrarily defined at 50% of distance from the center of tumor. Intensities of rVDI and rVSI were calculated by the ratios of core/rim. All data in this study were analyzed by Student's t-test. P value lower than 0,05 was considered statistically significant.

Results and Discussion

Tumor size was not significantly different between the control and the SCUBE3 knockdown transplants on day 28 (p=0.215) but the tumor size of SCUBE3 transplant was significantly smaller than the control after day 35(p<0.05) (Fig.1). With tumor growth, the amount of circulating tumor cells increased on day 35 and was higher in CL1-5 (shLacZ) than in CL1-5 (shSCUBE3) (Fig.2). The amount of circulating tumor cells is an index for cancer metastasis and is associated with the extent of tumor angiogenesis and vascularization (5-6). In this study, tumor angiogenesis and vascularization were evaluated by SSCE-MRI for tumors of a similar size. The rVDI and rVSI map showed a higher vascular density in the tumor core of the control CL1-5 (shLacZ) tumor while the tumor CL 1-5 (shSCUBE3) only showed high vascular density at rim (Fig.3(a)). The quantitative ratios of core/rim showed a lower vascular density in core than rim in the CL1-5 (shSCUBE3) knockdown tumor (Fig.3(b)). The shSCUBE3 knockdown inhibited the angiogenesis and vascularization in the tumor core and also limited the tumor growth and cancer cell invasion into the circulation system (6).

Conclusion

In this study, the SCUBE3-knockdown xenograft showed a less vascular penetration into the tumor core and suppressed the tumor growth. We conclude that SCUBE3 may play an important role in angiogenesis and tumor progression. It's a promising target for the subsequent cancer treatment studies.

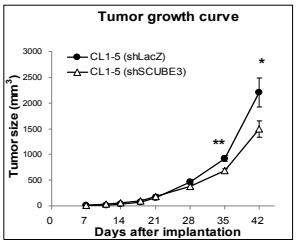

Fig.1 Tumor size was measured longitudinally after implantation The tumor growth is suppressed in SCUBE3 knockdown CL1-5 tumor. ∗, P < 0.05; ∗∗, P < 0.001. The number of repeats,*n* = 8 for CL1-5(shLacZ) tumors, and *n* = 7 for CL1-5 (shSCUBE3) tumors.

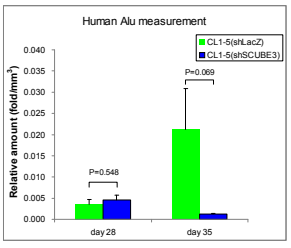

Fig.2 Quantitative measurement of circulating CL 1-5 tumor cells was performed by real-time qPCR for human *Alu* sequence. The relative amount of circulating tumor cells were normalized by the tumor size. The number of repeats, *n* = 7 for CL1-5(shLacZ) tumors, and *n* = 6 for CL1-5 (shSCUBE3) tumors).

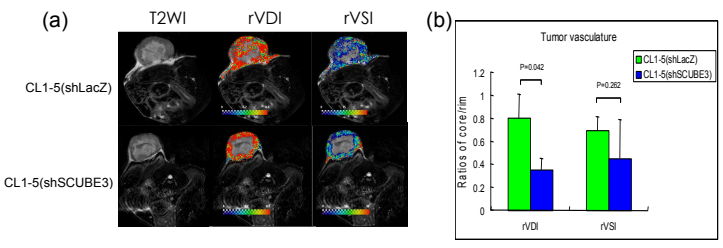

Fig.3(a) Relative vascular density and size are shown in rVDI and rVSI maps with T2WI as the reference. High rVDI was observed in CL1-5(shLacZ) in tumor core. (b)The ratios of core/rim on rVDI and VSI showed a significantly higher vascular density in CL1-5(shLacZ)Number of repeats, n=6, p=0.042), but no significant difference on the vascular size between CL1-5(shLacZ) and CL1-5(shSCUBE3) is observed..

References

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