Biodistribution and Gamma Camera Image of Anti-VEGF-2 Humanized Antibody, TTAC0001 in Xenografted Tumor Models.

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1. Introduction

Vascular endothelial growth factor (VEGF) and its receptors (VEGFR) have been implicated in promoting solid tumor growth and metastasis via stimulating tumor-associated angiogenesis [1-3]. Recently, anti-angiogenic therapies that interfere with the VEGF/VEGFR pathway may represent novel approaches to effective treatment. This study is aimed to evaluate tumor targeting of VEGFR-2 Ab in melanoma and chronic myelogenous leukemia (CML) tumor model for the feasibility of treatment using anti-VEGFR-2 antibody, TTAC-0001.

2. Methods and Results

2.1 RT-PCR analysis of VEGFR expression in human cell line.

Human A375 malignant melanoma and K562 chronic myelogenous leukemia cells were cultured in Dulbecco's Modified Eagle Medium and RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum, 1% antibiotics, at 37℃ in 5%CO_2. Total RNA was extracted using easy-spin™ Total RNA Extraction Kit (iNtRON Biotechnology). Synthesis of cDNA and PCR was performed from total RNA using OneStep RT-PCR kit (Qiagen), follwing the manufacturer's instructions. The VEGFR-2 primer used Human/Mouse VEGF R2 (KDR) Primer Pair (R&D). The β-actin primer sequences were as follows: Sense primer: GTG GGG CGC CCC AGG CAC CAG GGC; Antisense primer: CTC CTT AAT GTC ACG CAC GAT TTC.

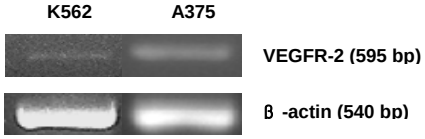


Fig.1 RT-PCR analysis for VEGFR-2 .

The expression of VEGFR-2 in A375 and K562 cells was analyzed by using VEGFR-2 and β -actin specific primers and was detected as 595 bp that amplified from cultured A375, K562 cells (Fig. 1).

2.2 Blood clearance of anti-VEGFR-2 Atibody.

Anti-VEGFR-2 Ab was labeled with iodine-131 using Iodobead method. Radiolabeling yield was analyzed by

ITLC-sg as a stationary phase and acetone as a mobile phase. The blood clearance of anti-VEGFR-2 Ab were performed with Balb/c mice injected via the tail vein, with ^{131}I-laveled anti-VEGFR-2 Ab at 5 mg /Kg from 10 minute to three day after injection. Blood weighed and counted in a gamma scintillation counter to determine the %ID/g. ^{131}I-VEGFR-2 showed fast clearance pattern and distribution and elimination half-life of anti-VEGFR-2 Ab were 0.27±0.02 h and 6.39±1.05 h, respectively (Fig.2).

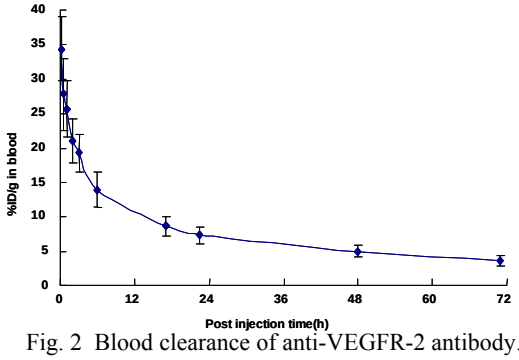
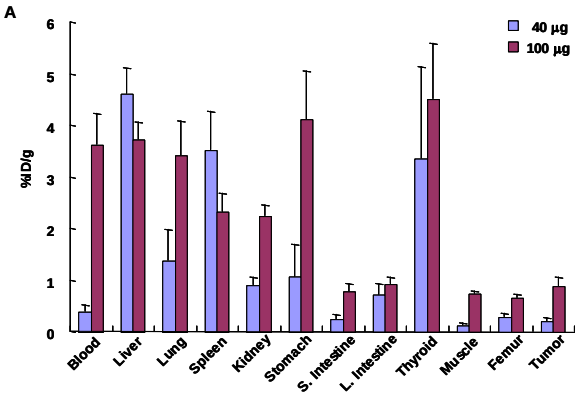


Fig. 2 Blood clearance of anti-VEGFR-2 antibody.

2.3 Biodistribution Studies of anti-VEGFR-2 antibody.

For biodistribution studies, 5 x 10^6 A375 cells and 1X10^7 K562 cells were implanted s.c. in female athymic mice. Tumor xenograft-bearing animals were used approximately 14 days after injection (tumor volume, 200~300 mm3). The biodistribution studies were performed with mice injected via the tail vein, with ^{125}I-labeled anti-VEGFR-2 antibody at 2 mg/Kg and 5 mg/Kg.



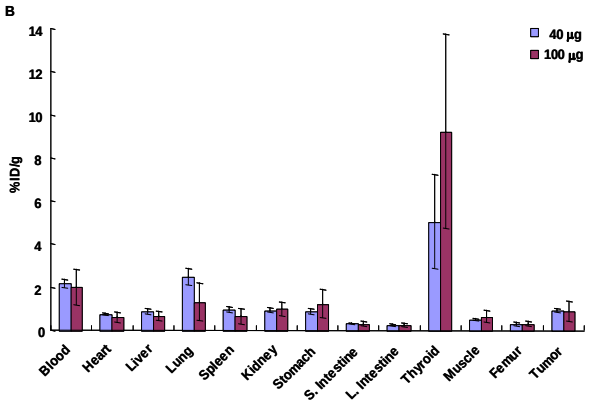


Fig. 3. Biodistribution of anti-VEGFR-2 antibody, TTAC-0001 in K562 (A) and A375 (B) tumor model.

After 24h, The tumor, blood, and major organs were removed, weighed, and counted in a gamma scintillation counter to determine the %ID/g. In case of K562, anti-VEGFR-2 Ab at 2 mg/Kg showed faster blood clearance (0.39±0.12 %ID/g vs. 3.63 ± 0.60 %ID/g) and lower tumor uptake (0.20±0.06 %ID/g vs. 0.89± 0.16 %ID/g) than at 5 mg/Kg. However, the ratios of tumor-to-blood (0.53±0.21 vs. 0.25±0.04) and tumor-to-muscle (1.67±0.80 vs. 1.19±0.22) at 2 mg/Kg were higher than those at 5 mg/kg (Fig.3A). In case of A375, tumor uptake of anti-VEGFR-2 Ab at 2 mg/Kg and 5 mg/Kg were 0.93±0.10 %ID/g and 0.87± 0.47 %ID/g, respectively. The ratios of tumor-to-blood (0.42±0.02 vs. 0.42±0.08) and tumor-to-muscle (1.88±0.10 vs. 1.50±0.76) at 2 mg/Kg were higher than those at 5 mg/kg (Fig. 3B).

2.3 Gamma camera images of anti-VEGFR-2 antibody

The Gamma camera images were performed with mice injected via the tail vein, with ^{131}I-labeled anti-VEGFR-2 antibody at 5 mg/kg.

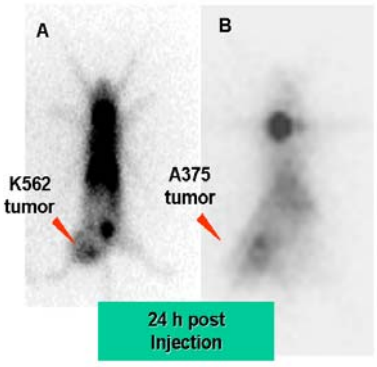


Fig. 4 Gamma camera images of anti-VEGFR-2 antibody at 24 h in K562(A) and A375(B) tumor models. Gamma camera images were obtained 30K counts with TRIONIX-XLT.

Radiolabeled anti-VEGFR-2 Ab was selectively localized in K562 amd A375 tumor (Fig. 4A and B).

3. Conclusions

In conclusion, A375 and K562 cells express VEGFR-2. Anti-VEGFR-2 Ab, TTAC-0001, showed VEGFR-2 specific tumor targeting in VEGFR-2 expressing tumor models. Anti-VEGFR-2 Ab could be used as a therapeutic agent for treatment.

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