Novel TNFR2 humanized mouse model for in vivo validation of human TNFR2 antibody targeting hTNF /hTNFR2 signaling pathways

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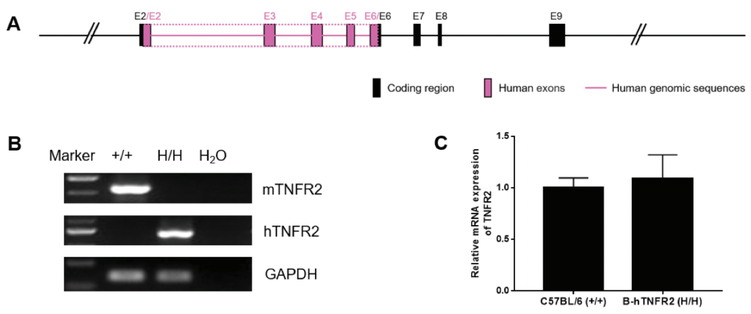
ABSTRACT

In recent years, immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment. Tumor necrosis factor receptor 2 (TNFR2), also known as tumor necrosis factor receptor superfamily member 1B (TNFRSF1B), is a transmembrane receptor that plays an essential role in immune modulation and tissue regeneration (Chen et al., 2008). TNFR2 is mainly expressed on the surface of immune cells in particular CD8 and CD4 T cells, including regulatory T cells in inflammatory and tumor-microenvironment (Wu et al, 2013, Azizi et al, 2018). TNFR2 promotes the proliferation of Tregs through nuclear factor kappa B (NF-κB) signaling (Rodriguez et al., 2011). TNFR2 also provided early costimulatory signals during CD4 and CD8 T cell activation (Kim et al, 2006). Both antagonistic and agonistic anti-TNFR2 antibodies have been developed to modify T cell functions and shown to inhibit tumor growth (Torrey et al, 2019, Tam et al, 2019). Therefore, TNFR2 has become an enthusiastically pursued therapeutic target for both immuno-oncology and autoimmune disease field.

To investigate the role of TNFR2, Biocytogen generated TNFR2 humanized mouse for both ***in vitro*** function validation of signaling pathway and *in vivo* efficacy evaluation of TNFR2 antibodies. In this model, the exons 2~6 of mouse *Tnfrsf1b* gene which encode the extracellular domain were replaced by human *TNFRSF1B* counterparts. Human extracellular domain of TNFR2 was detectable on the Tregs in spleen and the anti-human TNFR2 antibodies associated well to the splenocytes of the TNFR2 humanized mice. Basal leukocyte subpopulations of TNFR2 humanized mice were comparable to those of wild-type mice, including T/B cells, NK cells, DC, granulocytes and monocytes/macrophages. Anti-human TNFR2 antibodies bound with CD3+ T cells and inhibited tumor growth in TNFR2 humanized mice. Taken together, TNFR2 humanized mouse is a valuable tool for *in vivo* efficacy assessment of therapeutics that target human TNFR2.

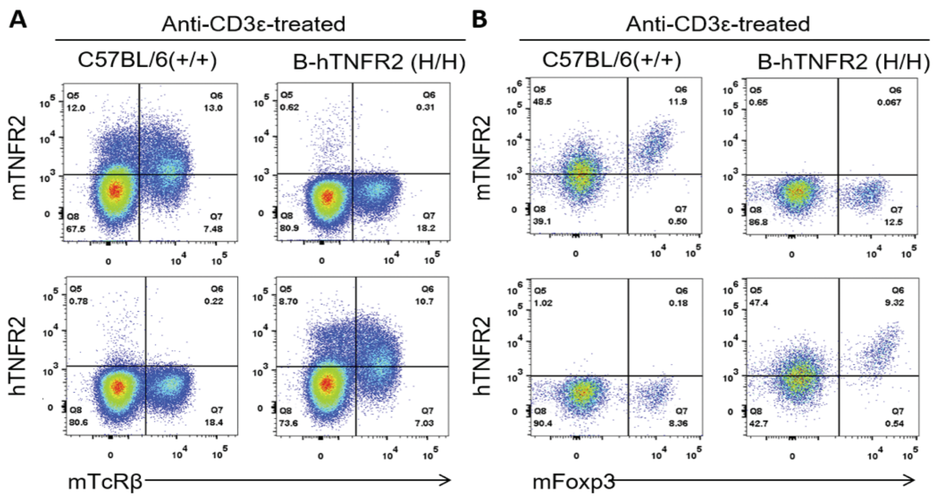
RESULTS AND DISCUSSIONS

Fig 1. Humanized TNFR2 mouse model generation and mRNA expression analysis



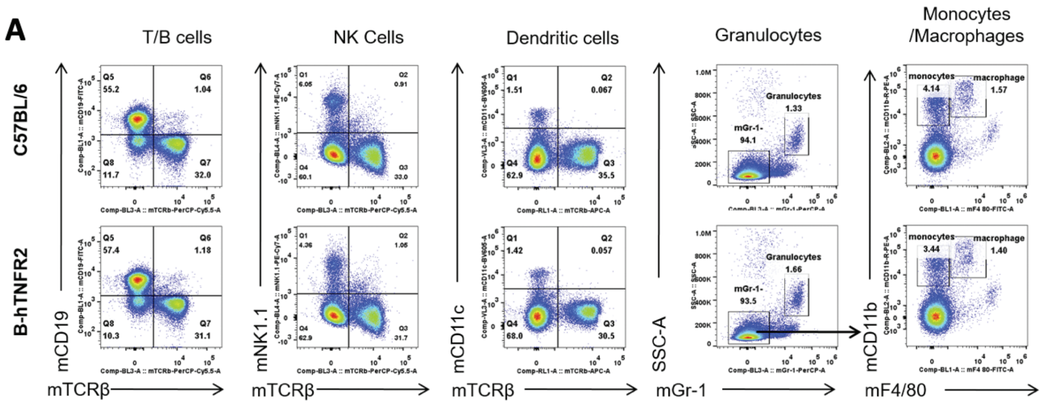
(A) Targeting strategy. (B) RT-PCR analysis of hTNFR2 expression. Mouse *Tnfrsf1b* mRNA was detectable only in splenocytes of wild-type (+/+) mice. Human *TNFRSF1B* mRNA was detectable only in H/H, but not in +/+ mice. (C) RT-qPCR analysis of hTNFR2 expression. The expression of hTNFR2 in B-hTNFR2 (H/H) was similar to that of mTNFR2 in the C57BL/6 (+/+) mice at mRNA level. Values were expressed as mean ± SEM.

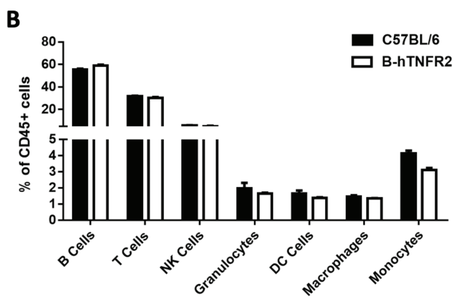
Fig 2. Protein expression analysis in B-hTNFR2 mice



Strain specific TNFR2 expression in homozygous B-hTNFR2 mice. Splenocytes were collected from WT and homozygous B-hTNFR2 (H/H) mice stimulated with anti-CD3ε *in vivo* and analyzed by flow cytometry with species-specific anti-TNFR2 antibody. Human TNFR2 was exclusively detectable in T cells (A) and Tregs (B) of homozygous B-hTNFR2 but not in WT mice.

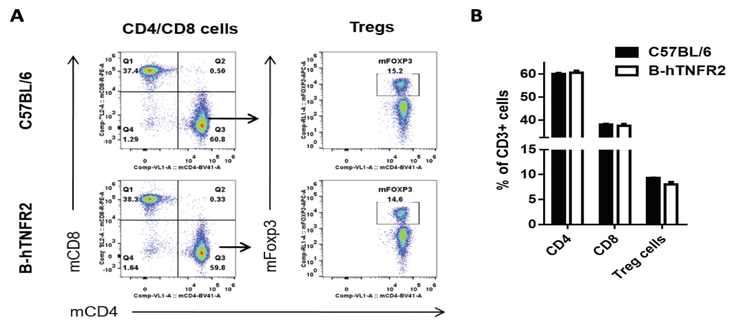
Fig 3. Analysis of spleen leukocytes cell subpopulations in B-hTNFR2 mice





Splenocytes were isolated from female C57BL/6 and B-hTNFR2 mice (n=3, 6-week-old). Flow cytometry analysis of the splenocytes was performed to assess leukocyte subpopulations. (A) Single live cells were gated for CD45 population and used for further analysis of leukocyte subpopulations as indicated in the representative FACS plots. (B) Results of FACS analysis. Percentages of T, B, NK, Monocyte, DC and macrophage cells in homozygous B-hTNFR2 mice were similar to those in the C57BL/6 mice, indicating that introduction of hTNFR2 extracellular domain in place of its mouse counterpart did not change the overall development, differentiation or distribution of these cell types in spleen. Values were expressed as mean ± SEM.

Fig 4. Analysis of spleen T cell subpopulations in B-hTNFR2 mice



Splenocytes were isolated from female C57BL/6 and B-hTNFR2 mice (n=3, 6-week-old). Flow cytometry analysis of the splenocytes was performed to assess T cell subpopulations. (A) Representative FACS plots. Single live CD45+ cells were gated for CD3 T cell population and used for further analysis as indicated. (B) Results of FACS analysis. Percent of CD8, CD4, and Treg cells in homozygous B-hTNFR2 mice were like those in the C57BL/6 mice, demonstrating that introduction of hTNFR2 extracellular domain in place of its mouse counterpart does not change the overall development, differentiation or distribution of these T cell sub types in spleen. Values were expressed as mean ± SEM.

Fig 5. Antibody binding assay in B-hTNFR2 mice

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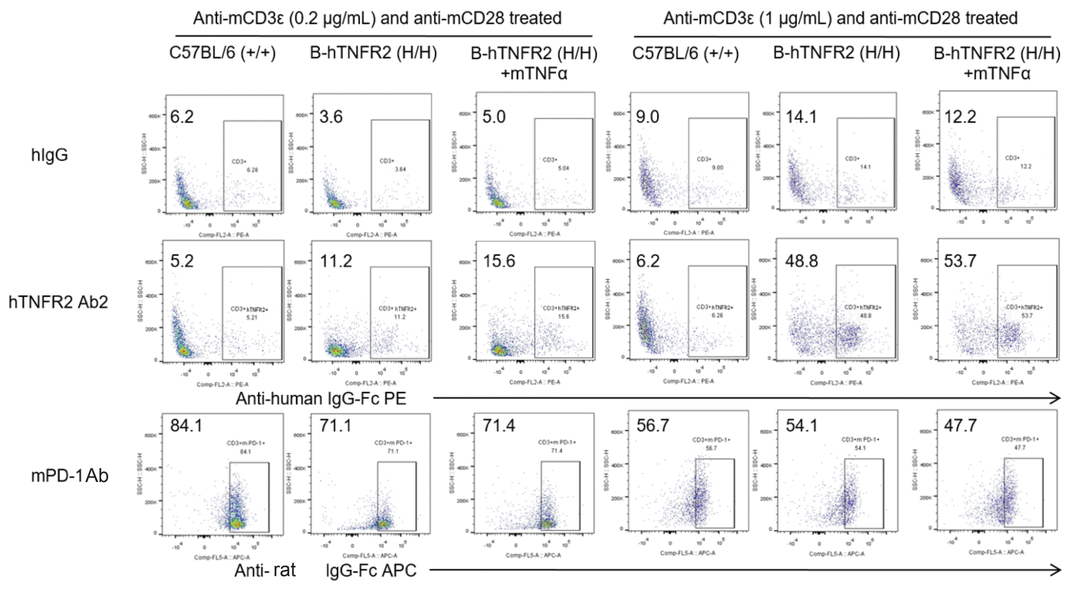

Binding of human anti-hTNFR2 antibody with activated T cells from B-hTNFR2 mice. Splenocytes were isolated from female B-hTNFR2 mice (n=3, 6-week-old) and treated with anti-mCD3ε (0.2 or 1 μg/mL) and anti-mCD28 (1 μg/mL) with or without mTNFa(200nM). Single live cells were gated for CD45+ population and used for further analysis as indicated. Strong human TNFR2 expression was detected by anti-hTNFR2 antibody in CD3+ T cells in B-hTNFR2 mice and the expression of hTNFR2 is further increased with mTNFa treatment. hIgG and mPD-1 Ab (RMP1-14) were used as control antibody. Anti-hTNFR2 antibody does not compete with mTNFa binding.

Fig 6. *In vivo* efficacy of anti-human TNFR2 antibodies

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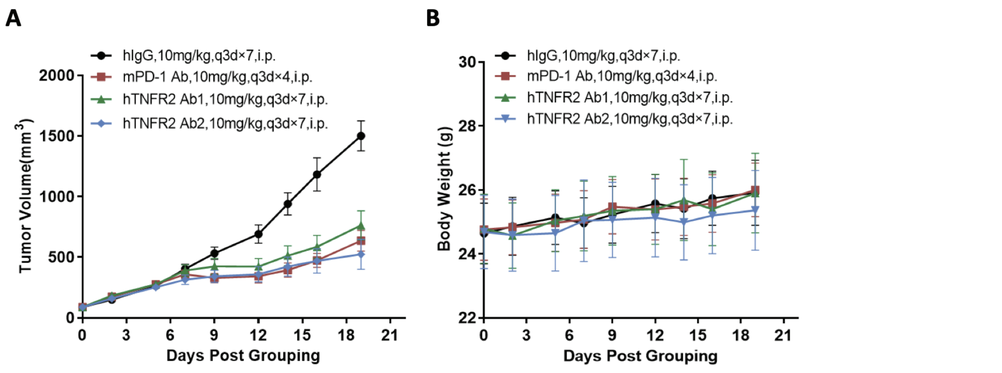

(A) Anti-human TNFR2 antibodies inhibited MC38 tumor growth in B-hTNFR2 mice. Murine colon cancer MC38 cells (5E5) were subcutaneously implanted into homozygous B-hTNFR2 mice (female, 9-10-week-old, n=8). Mice were grouped when tumor volume reached approximately 100 mm^3, at which time they were treated with anti-human TNFR2 antibodies with doses and schedules indicated in the panels. Anti-human TNFR2 antibodies were efficacious in controlling tumor growth in B-hTNFR2 mice. (B) Body weight changes during treatment. Values were expressed as mean ± SEM.

CONCLUSIONS

1. Humanized TNFR2 mouse model (B-hTNFR2) expresses TNFR2 protein that bears human TNFR2 extracellular domain with the mouse transmembrane and cytoplasmic domains.
2. B-hTNFR2 showed normal subpopulations of immune cells.
3. Anti-human TNFR2 antibodies showed robust anti-tumor efficacy in MC38/B-hTNFR2 mice.
4. The B-hTNFR2 mice are a powerful preclinical model for in vivo efficacy evaluation of human TNFR2 therapeutics.

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