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Construction of Siglec-15 knockout mouse model and validating the effect of Siglec-15 KO on T cell response

1634

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Background

Immune normalization cancer therapy, represented by anti-PD-1/PD-L1 treatment, have been proven effective in several cancer types and is revolutionizing cancer treatment. Several immune checkpoint blockers have been approved for treatment of certain cancers, which benefits a lot of patients with malignancies. However, the overall response rate to the current checkpoint blockers does not exceed 30% in many cases, stressing the importance of investigation and development of new immunotherapies.

Sialoglycans are sialic acid sugar-carrying glycans expressed on mammalian cells to help evade self immune responses by engaged with the inhibitory Siglecs (sialic acid-binding immunoglobulin-like lectins) on immune cells. Aberrant hypersialylation has been reported in many tumor cells and significantly influences tumorigenesis and cancer progression by contributing to an immunosuppressive tumor microenvironment (TME). Thus therapeutic approaches targeting either sialologlycans or Siglecs will be promising for immune normalization therapy.

Siglec-15 is reported by Pr. Lieping Chen's lab to suppresses antigen-specific T cell responses in vitro and in vivo. Genetic ablation or antibody blockade of Siglec-15 amplifies anti-tumor immunity in the TME and inhibits tumor growth in some mouse models. Clinical trial to test the effect of a humanized mAb (NC318) to Siglec-15 in solid tumors is ongoing.

In order to establish a platform to study the effect of potential Siglec-15 antibodies or inhibitors, we first generated Siglec-15 KO mice with CRISPR/Cas9 mediated genome editing. We tested tumor establishment on this mice with syngeneic cell lines and results showed that Siglec-15 KO inhibits syngeneic tumor growth, supporting the role of Siglec-15 as a promising target for development of new immune checkpoint blocker. We also tested the effect of Siglec-15 KO for macrophages to stimulated T cell response in vitro and in vivo but the results showed no dramatic difference. In summary we constructed a Siglec-15 KO mouse model and we will keep testing the effect of Siglec-15 KO on tumor immunotherapy with this model.

Results

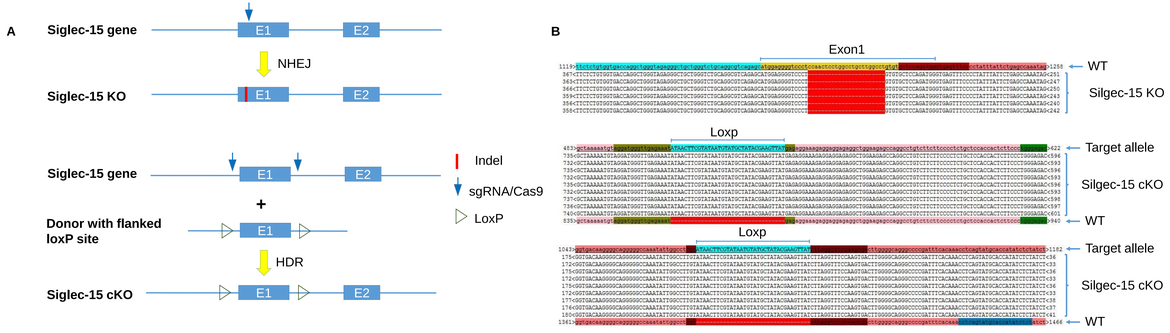

Figure 1 Strategy for construction of Siglec-15 KO and cKO mouse.

A. Schematic strategy for construction of Siglec-15 KO and cKO mouse model with CRISPR/Cas9. For Siglec-15 KO, sgRNA was designed targeting Exon1 and Siglec-15 KO was realized by indels from NHEJ. For Siglec-15 cKO, Loxp sites were inserted flanking Exon1 by HDR and will results in Exon1 deletion by breeding with Cre mice.

B. Sequencing results for Siglec-15 KO and cKO homozygous mice. For Siglec-15 KO, deletion of 23bp in Exon results in frameshift and premature stop codon. For Siglec-15 cKO, LoxP sites were successfully inserted flanking Exon1.

Figure 2 *In vivo* tumor growth assay of MC38 with or without human SIGLEC15 overexpression

A. WT or Siglec-15 KO mice were inoculated subcutaneously with MC38 WT or MC38 overexpressing human Siglec-15 and tumor volume were recorded. Results showed that Siglec-15 KO inhibits MC38 tumor growth but human Siglec-15 overexpression in MC38 also decrease tumor growth, which will be further verified.

B. qPCR analysis of human Siglec-15 mRNA expression in MC38 cells. WB failed to detect human SIGLEC-15 protein in the transduced CT26 or MC38 cells with several available antibodies though strong mRNA expression was detected by qPCR.

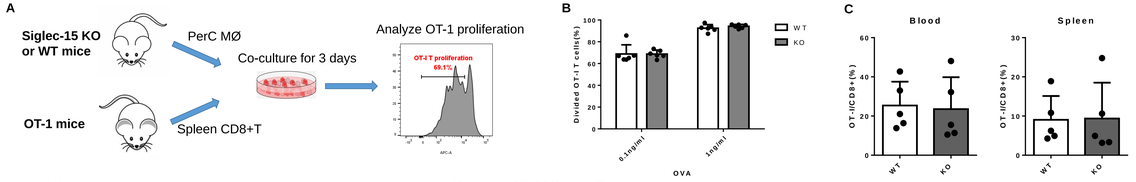

Figure 3 *In vitro* and *in vivo* assay to test the effect of Siglec-15 KO on T cell response

A. Illustration of the In vitro study process. Peritoneal Cavity macrophages (PerC MØ) were collected from Siglec-15 KO or WT mice 4 days after the mice were inject with 2 ml of 3% (w/v) Brewer thioglycollate. Cd8+ T cells were isolated from spleencytes of OT-1 mice, stimulated with OVA peptide and labeled with CellTrace. The labeled Cd8+ T cells were co-cultured for with the PerC MØ for 3 days before cell proliferation were analyzed .

B. The ratio of divided OT-1 T cells after co-cultured with Siglec-15 KO or WT PerC MØ for 3 days stimulated with different concentration of OVA peptide.

C. *In vivo* OT-1 T cell response to OVA stimulation in WT or Siglec-15 KO mice. WT or Siglec-15 KO mice were injected with OT1 T cells intravenously on Day0 and immunized with OVA peptide plus poly(I:C) intraperitoneally on Day1. OT-1 T/Cd8+ T ratio in blood and spleen were analyzed by flow cytometry on Day6.

Summary and Discussion

1. We successfully constructed Siglec-15 KO and cKO mouse model, verified by sequencing of the target region in Siglec-15 gene.
2. We tested MC38 tumor growth in the constructed Siglec-15 KO mice and found that Siglec-15 KO inhibit tumor growth, which is consistent with the reports.
3. We constructed CT26/MC38 cell lines overexpressing human Siglec-15 but failed to detect SIGLEC-15 protein expression, even with treatment of proteasome inhibitor. We will try SIGLEC-15 overexpression in other cell lines and test alternative antibodies.
4. We tested the effect of Siglec-15 KO on T cell response in vitro and in vivo but found no obvious influence. We will keep on validating the model and verifying the effect of Siglec-15 KO on tumor growth and immune response with more assays.

Reference

- Wang, J., Sun, J., Liu, L.N. *et al.* Siglec-15 as an immune suppressor and potential target for normalization cancer immunotherapy. *Nat Med* **25,** 656–666 (2019).
- van de Wall S, Santegoets KCM, *et al.* Sialoglycans and Siglecs Can Shape the Tumor Immune Microenvironment. *Trends Immunol*. 2020 Apr;41(4):274-285.