GPR65 impeded intestinal inflammation and colitis-associated colorectal cancer development in experimental murine models

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Background
G-protein coupled receptors are the largest group of pharmacologically targeted receptors. GPR65 (also known as T-cell death-associated gene B, TDAOB) is a proton sensing receptor predominantly expressed on immune cells. Genome-wide association study (GWAS) identified GPR65 gene polymorphisms as a potential risk factor in inflammatory bowel disease (IBD) patients. Like patients are at a higher risk of developing colorectal cancer (CRC) than the general population.

Methods
To establish the chronic colitis mouse model, wild-type (WT) (n=15) and GPR65−/− (n=15) mice were administered 3% DSS for four (5 days) cycles in drinking water, integrated by 2 days of water-only remission cycles. Following 4th cycle water was switched back to 3% DSS for 2 final days, then mice were euthanized. Real-Time PCR using Taqman pre-designed primer for p-actin and GPR65 was performed for Unbiased Colitis (UC) and Crohn’s Disease (CD) patients’ samples. For the colitis associated colorectal cancer (CAC) model to be established, WT (n=21) and GPR65−/− (n=21) mice were administered one dose of AOM (10mg/kg) followed by three (5 days) cycles of oral administration of 4% DSS integrated by water-only recovery cycles. Mice were euthanized between 13-14 weeks post-treatment for tissue collection and tumor assessment.

Hypothesis

H+ (protons)

GPR65 (TDAG8)

Leukocyte Inflammation

Cytokines

Inflammation (IBD)

Dysplasia

Colitis Associated Colorectal Cancer (CAC)

Figure 1. GPR65 activation in immune cells by protons resulting in a wide range of inflammatory responses, leukocyte (immune cell infiltration) and cytokine production. This in turn fuels inflammatory bowel disease' inflammation which increases the risk to develop colitis associated colorectal cancer.

Figure 2. 4Dx showing leukocyte infiltration of WT and GPR65 KO mice. (A) H&E for CD4+ cells, WT DSS: B: GPR65- KO DSS: and (B) Quantification of leukocytes in dextran colitis. Mann Whitney Student t-test (P<0.001).

Figure 3. Chronic DSS colitis clinical phenotype and macroscopic disease indicators of WT and GPR65 KO mice: (A) body weight loss, (B) ileal blood score, (C) ileal histological lymph node enlargement, and (D) colon shortening. One way ANOVA (**P<0.01, ***P<0.001).

Figure 4. 4Dx showing leukocyte infiltration of WT and GPR65 KO mice. (A) H&E for CD4+ cells, WT DSS: B: GPR65- KO DSS: and (B) Quantification of leukocytes in dextran colitis. Mann Whitney Student t-test (P<0.001).

Figure 5. CD3+ showing leukocyte infiltration of WT and GPR65 KO mice. (A) H&E for CD4+ cells, WT DSS: B: GPR65- KO DSS: and (B) Quantification of leukocytes in dextran colitis. Mann Whitney Student t-test (P<0.001).

Figure 6. Human GPR65 mRNA expression in lesions from colitis and Crohn’s patients. GPR65 mRNA expression in (A) human colitis, and (B) human Crohn’s disease, both compared to normal tissue. Mann Whitney students t-test (P<0.001) and (B) Quantification of mRNA volume. Mann Whitney student t-test (P<0.001).

Figure 7. 4Dx showing leukocyte infiltration of WT and GPR65 KO mice. (A) H&E for CD4+ cells, WT DSS: B: GPR65- KO DSS: and (B) Quantification of leukocytes in dextran colitis. Mann Whitney Student t-test (P<0.001).

Figure 8. CAC clinical phenotype and macroscopic disease indicators of WT and GPR65 KO mice: (A) body weight loss, (B) sporadically, (C) CD4+ mesenteric lymph node enlargement, (D) ileal inflammation (GCF), and (E) Quantification of leukocytes in dextran colitis. Mann Whitney Student t-test (P<0.001).

Figure 9. Polypos number and volume in WT and GPR65 KO-JAMO/DSS mice. (A) picture of distribution in WT-JAMO/DSS mouse panel and GPR65- KO-JAMO/DSS (lower panel). (B) Quantification of polypos number and (C) Quantification of polypos volume. Mann Whitney student t-test (P<0.001).


Figure 11. Ablation and infiltration around tumor area. (A) H&E, strom polyps of WT and GPR65 KO-JAMO/DSS (4X magnification). A. WT, B. GPR65 KO, (B) Infiltration immune score. Mann Whitney Student t-test shows (P<0.001).

Figure 12. C4dx showing immune infiltration of WT and TDAG KO mice. (A) H&E for CD4+ cells, WT-JAMO/DSS: B. GPR65-KO-JAMO/DSS: and (B) Quantification of immune infiltration. Mann Whitney student t-test (P<0.001).

Conclusion
Our data demonstrate that GPR65 suppresses intestinal inflammation and colitis-associated tumor development in the mouse models suggesting that potentiation of GPR65 with agonists may have anti-inflammatory therapeutic effects in IBD and reduce the risk of developing colitis-associated colorectal cancer.

References

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