GPR65 impedes 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 8, TDAG8) 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. IBD 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=13) and GPR65 -/- (n=13) 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 probe for β-actin and GPR65 was performed for Ulcerative 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 *i.p.* (10mg/kg) followed by three (5 day) 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

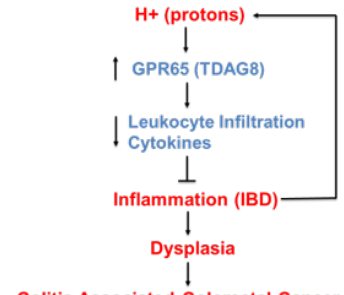

Figure 1: GPR65 activation on immune cells by protons resulting from acidic environment of inflammation, downregulates leukocyte (immune cell infiltration) and cytokines production. This in turn halts inflammatory bowel disease/ inflammation which reduces the risk to develop colitis associated colorectal cancer.

Results

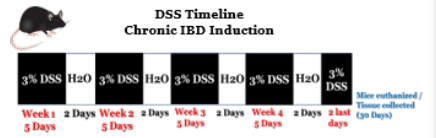

Figure 2: IBD induction timeline in WT and GPR65 KO mice.

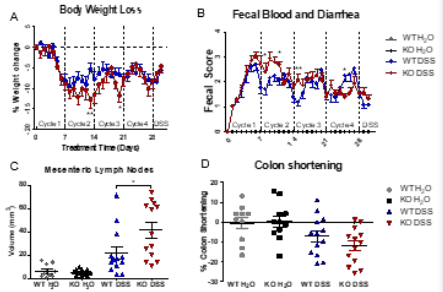

Figure 3: Chronic DSS colitis clinical phenotype and macroscopic disease indicators of WT and GPR65 KO-DSS mice. (A) Body weight loss, (B) fecal blood score, (C) mesenteric lymph node enlargement, and (D) colon shortening. One Way ANOVA (* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001).

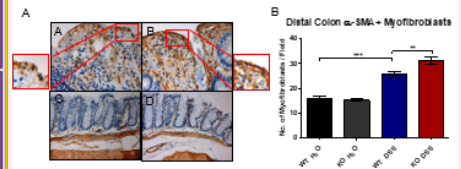

Figure 4: α-Smooth Muscle Actin (α-SMA) showing Myofibroblast activation of WT and GPR65 KO mice distal colons. (A) IHC for α-SMA+ cells (40X magnification), A: WT-DSS, B: GPR65 KO-DSS, C&D: WT & TDAG8 KO-control, and (B) Quantification of myofibroblasts in distal colon. One way ANOVA (** P ≤ 0.01, *** P ≤ 0.001).

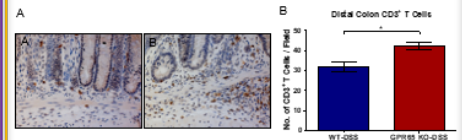

Figure 5: CD3+ showing leukocyte infiltration of WT and TDAG8 KO mice distal colons. (A) IHC for CD3+ cells, A: WT-DSS, B: GPR65 KO-DSS, and (B) Quantification of leukocytes in distal colon. Mann Whitney student t-test (* P ≤ 0.05).

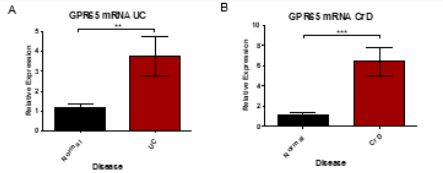

Figure 6: Human GPR65 mRNA expression in lesions from colitis and Crohn's samples. GPR65 mRNA expression in (A) human colitis, and (C) human Crohn's disease, both compared to normal tissue. Mann Whitney students t-test (** P ≤ 0.01 and *** P ≤ 0.001).

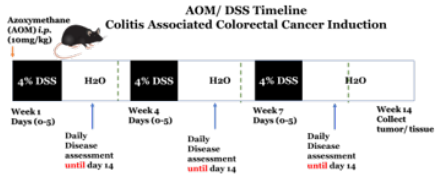

Figure 7: IBD induction timeline in WT and GPR65 KO mice.

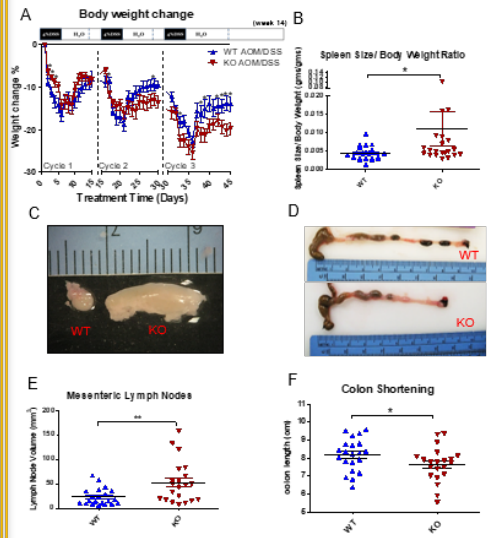

Figure 8: Colitis associated colorectal cancer (CAC) clinical phenotype and macroscopic disease indicators of WT and GPR65 KO- AOM/DSS mice. (A) Body weight loss, (B) splenomegaly, (C&E) mesenteric lymph node enlargement, (D&F) colon shortening. (* P ≤ 0.05, ** P ≤ 0.01).

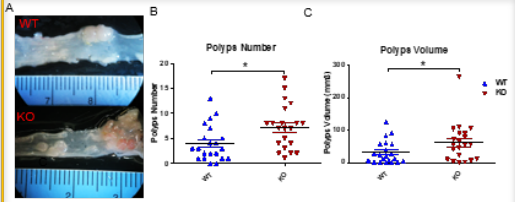

Figure 9: Polyps number and volume in WT and GPR65 KO-AOM/DSS mice colons. (A) picture of distal colon in WT-AOM/DSS (upper panel) and GPR65 KO-AOM/DSS (lower panel). (B) Quantification of polyps number and (C) Quantification of polyp volume. Mann Whitney student t-test (* P ≤ 0.05).

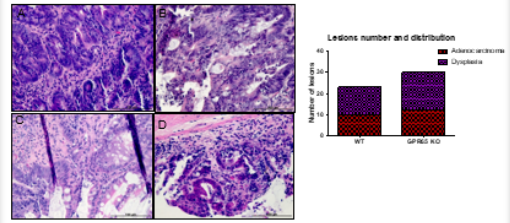

Figure 10: Dysplasia and adenocarcinoma lesions and distributions. (A) H&E stain polyps of WT and GPR65 KO-AOM/DSS (40X magnification), A: WT-AOM/DSS dysplasia, B: GPR65 KO-AOM/DSS dysplasia, C: WT-AOM/DSS adenocarcinoma, D: GPR65 KO-AOM/DSS adenocarcinoma (B) Quantification of dysplasia and adenocarcinoma lesions.

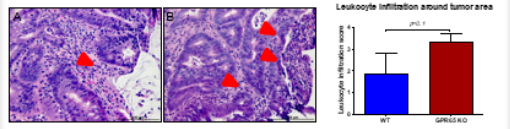

Figure 11: Immune cell infiltration around tumor area. (A) H&E stain polyps of WT and GPR65 KO-AOM/DSS (40X magnification), A: WT, B: GPR65 KO, (B) Immune infiltration score. (Mann Whitney students t-test shows *p*=0.1).

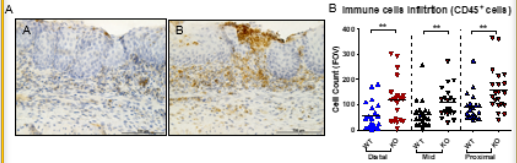

Figure 12: CD45+ showing Immune infiltration of WT and TDAG8 KO-AOM/DSS mice colons. (A) IHC for CD45+ cells, A: WT-AOM/DSS, B: GPR65 KO-AOM/DSS (40X magnification), and (B) Quantification of immune in distal, mid and proximal colon sections. Mann Whitney student T-test (** P ≤ 0.01).

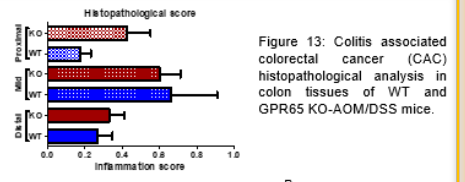

Figure 13: Colitis associated colorectal cancer (CAC) histopathological analysis in colon tissues of WT and GPR65 KO-AOM/DSS mice.

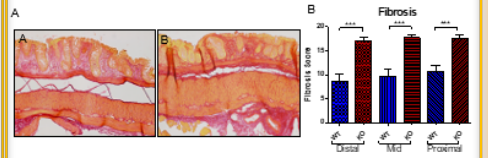

Figure 14: Fibrosis in colitis associated colorectal cancer (CAC) in colon tissues of WT and GPR65 KO-AOM/DSS mice. (A) picrosirus red stain (collagen stain) in distal colon, A: WT-AOM/DSS, B: GPR65 KO-AOM/DSS, and (B) fibrosis score in distal, mid, and proximal colons. Mann Whitney (* P ≤ 0.001).

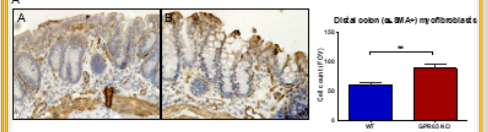

Figure 15: α-Smooth Muscle Actin (α-SMA) showing Myofibroblast activation of WT and TDAG8 KO mice distal colons. (A) IHC for α-SMA+ cells (40X magnification), A: WT-AOM/DSS, B: GPR65 KO-AOM/DSS, and (B) Quantification of myofibroblasts in distal colon. Mann Whitney students t-test (** P ≤ 0.01).

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

1- Rosenbaum et al, Nature, 2009, 459(7245):p.356-363.
2- Franke et al., Nat. Genet., 2010. 42(12):p.1118-25.
3- Jess T. et al., Clinical Gastroenterol. Hepatol., 2012, 10(6):639-45.

Acknowledgement

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