ACTIVATION OF FXR RESTORES EXPRESSION OF GENES DYSREGULATED IN HUMAN IBD AND SUPPRESSES TNFα AND IL-6 SIGNALING PATHWAYS IN ADOPTIVE TRANSFER COLITIS MODEL

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INTRODUCTION

Farnesoid X receptor (FXR) is a ligand-activated nuclear hormone receptor that is highly expressed in the liver and gastrointestinal tract. FXR agonists are in development for the treatment of nonalcoholic steatohepatitis (NASH) and other hepatobiliary diseases. We have previously shown our FXR agonist M480 demonstrates efficacy in multiple chronic IBD models (adoptive transfer (AT) colitis, Mdr1a$^{-/-}$ colitis, and SAMP1/YitFc ileitis) with efficacy similar to that of anti-IL-12/23. Similar efficacy has been observed with our clinical FXR agonists MET409 and MET642. To investigate the mechanisms by which FXR agonists reduce colitis and explore the relevance to human IBD, we identified the genes and pathways regulated by FXR in the AT and Mdr1-/- colitis mice, and compared them with the gene expression profile in human IBD patients.

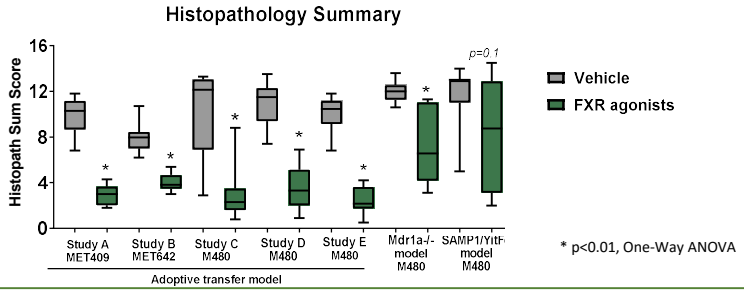
MATERIAL & METHODS

Colitis was induced by transferring CD4+CD45RB^{hi} T-cells to C.B-17 SCID mice. All treatments were in therapeutic mode, started 21 days post AT, or beginning when disease is present at week 8-9 in Mdr1a$^{-/-}$ mice, and lasted for 4 weeks: FXR agonist MET409 (10mg/kg, QD), MET642 (0.3mg/kg, QD), or M480 (10mg/kg, QD), n=10 for each treatment. Anti-IL-12p40 (0.5mg/mouse) was dosed i.p. weekly (n=5). Efficacy was assessed by colon histopathology. RNAseq was performed on colon RNA (n=5-7/group). Genes differentially regulated by M480 were compared with published FXR ChIPSeq data (Ref. 2) and a IBD patients' gene expression dataset (GSE73661, Ref.1). Gene set enrichment analysis (GSEA) was performed to identify enriched pathways.

RESULTS

FXR Agonists Improve Colon Histopathology in Multiple IBD Models

- Three chronic IBD models used in therapeutic treatment mode
  - T-Cell Adoptive Transfer
  - Mdr1-/-
  - SAMP1/YitFc
- Reproducible efficacy observed with multiple Metacrine FXR agonists
  - MET409 and MET642 are currently in phase II trials for NASH
  - M480 proof of concept compound

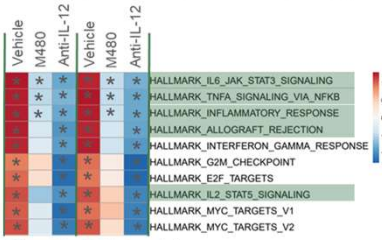


* p<0.01, One-Way ANOVA

FXR Activation Suppresses TNFα and IL-6 Signaling Pathways

- RNAseq analysis was performed on colons from AT and Mdr1-/- studies
- GSEA was performed on the mouse RNAseq data and human IBD GSE73661 data with infliximab (IFX) treatment responders (Ref.1) to identify pathways similarly regulated in mouse and human IBD
- TNFα and IL-6 signaling are among the top pathways similarly up-regulated in both human and mice with IBD.
  - Both pathways are down regulated by FXR activation by M480 or anti-IL12 in the mouse models
  - These pathways are both targeted by therapeutics approved for or in development for IBD

Top 10 pathways dysregulated in mouse IBD & reversed by FXR activation or anti-IL12 antibody

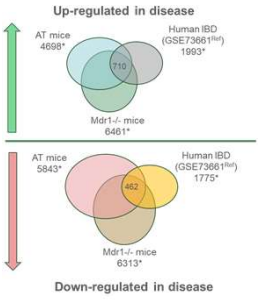


Top 10 pathways dysregulated in IBD patients and reversed by infliximab

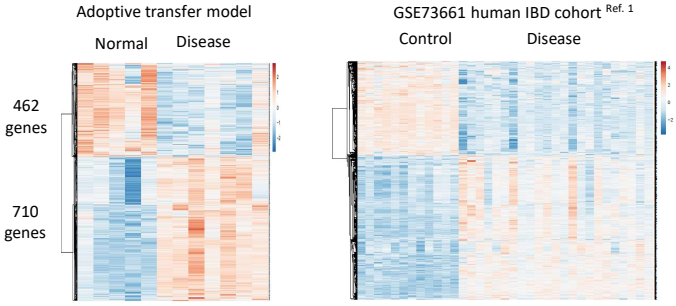
Green highlights similar pathways between mice and human
* FDR <0.05, geneset permutation; # FDR <0.2, phenotype permutation

Identification of Genes Similarly Dysregulated in Human and Mouse IBD

- To identify genes dysregulated in diseased vs healthy control in the AT, Mdr1-/- and patients with Ulcerative Colitis (GSE73661), RNAseq data was examined using limma analysis for differentially expressed genes with an FDR of 0.05
- We identified over 1000 genes similarly dysregulated in humans and mice with IBD: 462 up-regulated and 710 down-regulated

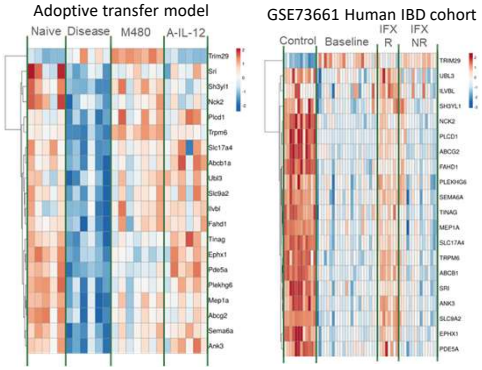


* Number of differentially expressed genes versus normal control



Identification of 20 Potential Direct FXR Target Genes Dysregulated in Human IBD

- Genes dysregulated in human and mouse IBD (identified above), were examined for FXR response elements identified by FXR ChipSeq studies (Ref.2)
- Identified 20 potential FXR target genes, many with functions implicated in IBD



IFX R – responder
IFX NR – non-responder

Genes with functions associated with IBD

Genes	Function
ANK3, SLC17A4, SLC9A2, SRI, TRPM6	Gut transporter function
ABCB1A, ABCG2, PLEKHG6	Gut barrier function
SEMA6A, SRI, TRIM29	Modulates inflammation
MEP1A	Promotes remodeling of injured gut
EPHX1	Detoxification

CONCLUSIONS

Comparison of RNAseq data from AT and Mdr1-/- colitis models with IBD patients shows pathways and genes similarly dysregulated in mice and humans with IBD. While not a direct immune modulator, FXR activation can decrease TNFα and IL-6 signaling pathways. Since FXR is a transcriptional activator, these effects likely occur indirectly and downstream of FXR target gene activation. We identified 20 potential FXR target genes with possible roles in IBD in both humans and mice. These findings support further development of novel FXR agonists as a potential oral therapy for IBD.

References

1. Arijs I, et al. Effect of vedolizumab (anti-α4β7-integrin) therapy on histological healing and mucosal gene expression in patients with UC. *Gut* 2018 Jan;67(1):43-52
2. Thomas et al., Genome-Wide Tissue-Specific Farnesoid X Receptor Binding in Mouse Liver and Intestine. Hepatology. 2010 Apr; 51(4): 1410–1419

DISCLOSURES

B.W., A.M., X.L. are employees and equity holders in Metacrine, Inc.