# Ulcerative Colitis: The MeCP2/H3K4me3 interaction in the hypermethylated promoter modulates NGF gene expression

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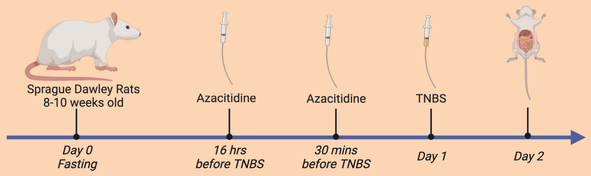
## INTRODUCTION

- **Chronic inflammatory bowel disease, such as Ulcerative colitis, is characterized by abdominal pain with limited pharmacological therapies.**
- **Approximately 1 million Americans are diagnosed with UC every year, according to the CDC.**
- **The underlying mechanisms of colitis are complex due to an excessive and exacerbated immune response within the gastrointestinal tract.**
- **Nerve growth factor (NGF) is significantly elevated in several inflammatory conditions, including UC, and is essential for a robust inflammatory response.**
- **Epigenetic processes like DNA methylation and histone modification play an important role in the epigenetic regulation of key biomarkers in developing several inflammatory conditions including UC.**
- **The cross talk between epigenetic factors, Methyl-CpG-binding protein 2 (MeCP2) and tri-methylation of lysine 4 on histone H3 (H3K4me3) have been shown to be involved in the regulation of gene expression.**
- **However, very little is known regarding how NGF signaling is modulated in UC through epigenetic mechanisms.**
- **This study gave the better understanding about the integrated role of DNA methylation, MeCP2 and histone modifications in regulating the NGF gene in experimental colitis.**

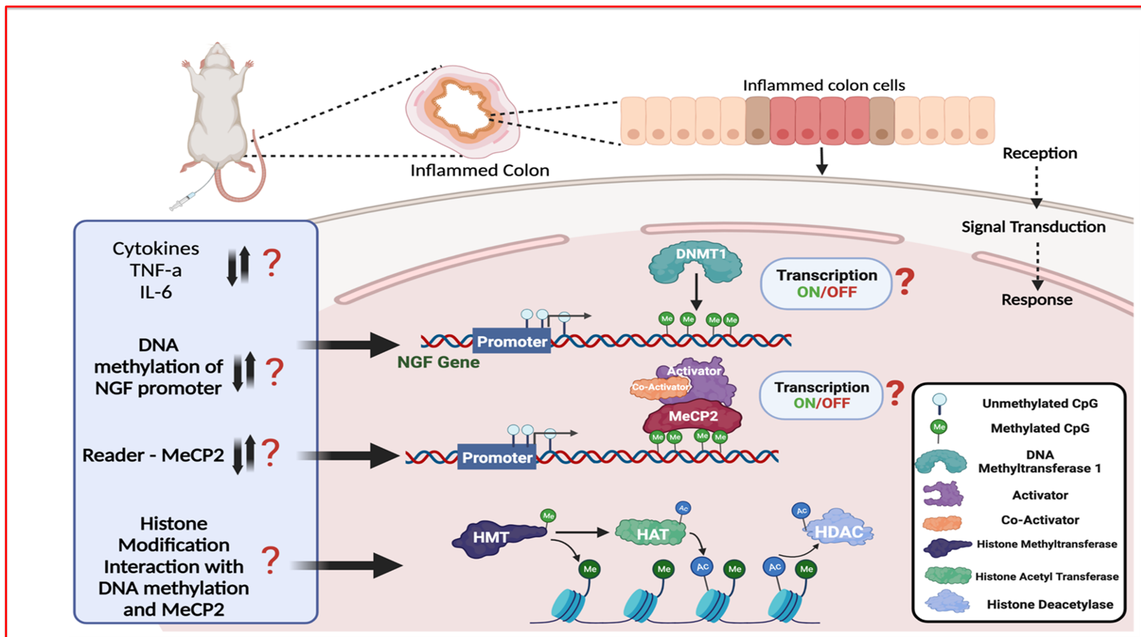
## AIM

**In this study, we determined the MeCP2 interaction with histone modification (H3K4me3) for epigenetic regulation of the NGF gene during colonic inflammation.**

## METHODS




| Techniques | Application |
|---|---|
| Conventional PCR<br>Real Time qPCR | Determination and quantitation of primary transcript (NGF and MeCP2) |
| Western Blot analysis | Qualitative and quantitative analysis of proteins (NGF and MeCP2) |
| Bisulfite Conversion **(BC)** and Methylation Specific PCR **(MSP)** | DNA methylation Status of NGF gene |
| Chromatin immunoprecipitation **(ChIP)** | Protein (Histone modification and MeCP2) – gene(NGF) interaction |
| Immunoprecipitation **(IP)** | Protein (MeCP2) and Protein (Histone modification H3K4me3) |




During the colonic inflammatory process, the regulation of the *NGF* gene is controlled by simultaneous interaction of epigenetic mechanisms like DNA methylation, histone modifications and methyl-CpG-binding protein2

## RESULTS

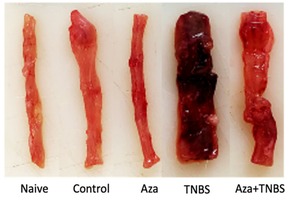



Fig 1: TNBS treatment induced local colon inflammation, while Aza pretreatment reduced TNBS-induced inflammation

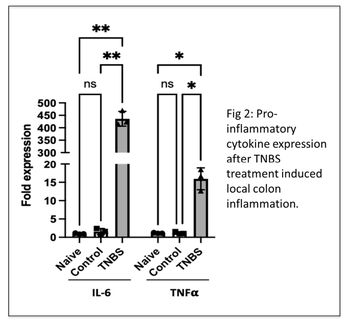



Fig 2: Pro-inflammatory cytokine expression after TNBS treatment induced local colon inflammation.

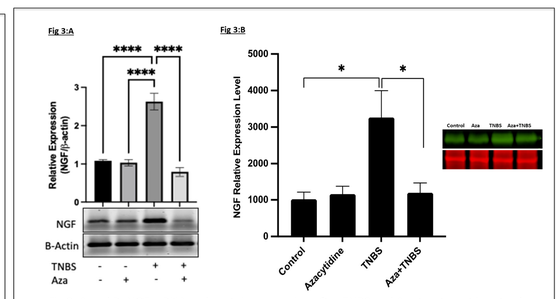



Fig 3: A and B: NGF mRNA and protein expression after TNBS and Aza treatment in rat colon.

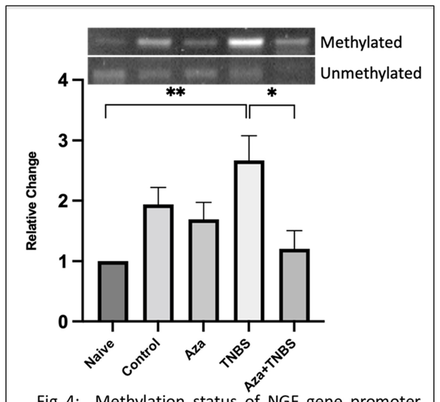



Fig 4: Methylation status of NGF gene promoter region (Specific Methylated and unmethylated primers were used).

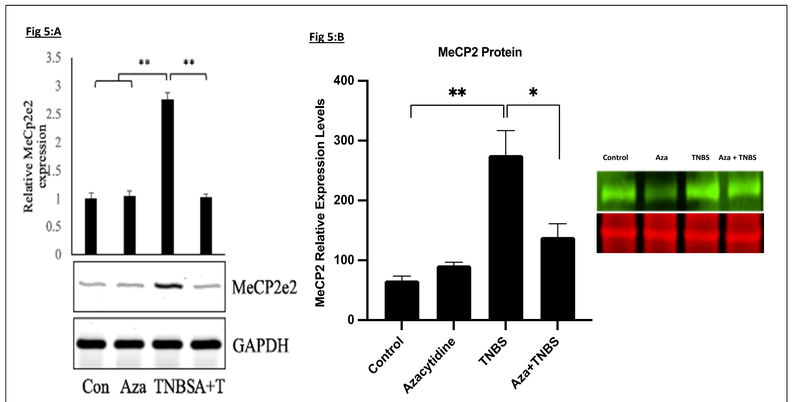



Fig 5: A and B: MeCP2 mRNA and protein expression after TNBS and Aza treatment in rat colon.

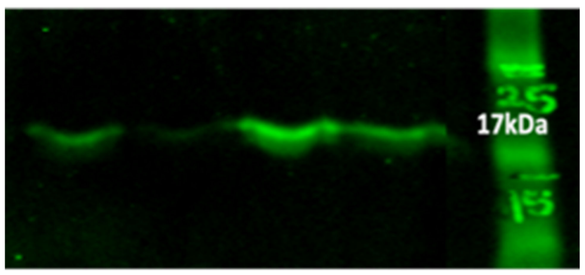



Fig 6: H3K4me3 expression in nuclear fraction

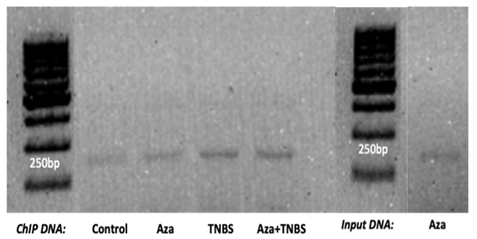



Fig 7 : Chromatin immunoprecipitation (ChIP) assay: Association of H3K4me3 and NGF gene promoter region. H3K4me3 antibody used to pull down NGF gene promoter region.

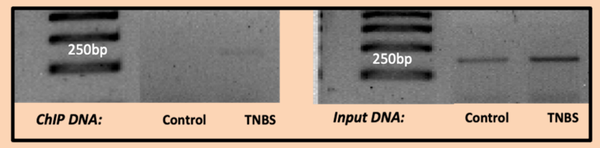



Fig 8 : Chromatin immunoprecipitation (ChIP) assay: Association of MeCP2 and NGF gene promoter region. MeCP2 antibody used to pull down NGF gene promoter region.

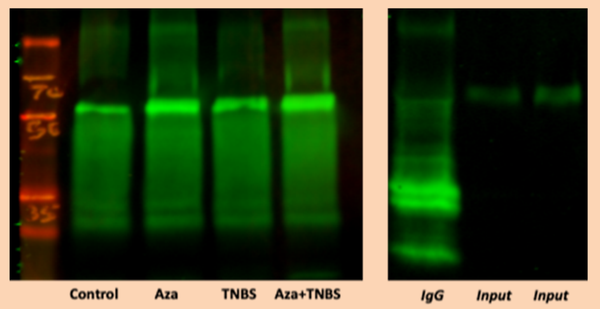



Fig 9 : Immunoprecipitation (IP) assay:MeCP2 protein pulled down using H3K4me3 antibody

## CONCLUSION

- **Our findings showed increased NGF expression in the colon during TNBS induced colitis due to hypermethylation of CpG dinucleotides in the *NGF* promoter.**
- **Aza treatment mitigated hypermethylation and reduced neurogenic inflammation in these animals, suggesting involvement of epigenetic regulation of *NGF* in neuroinflammation.**
- **MeCP2 is upregulated in TNBS-treated animals. MeCP2 recruitment to the *NGF* promoter was altered compared to control animals during colon inflammation.**
- **Our results suggest a strong interaction between MeCP2 and H3K4me3 in regulation of NGF expression during colitis while Aza pretreatment disrupts this interaction.**
- **Thus, we conclude that TNBS treatments could induce the crosstalk between histone modifications, DNA methylation and MeCP2, thus regulating *NGF* gene expression.**

## FUTURE DIRECTIONS

- **Evaluate the translational efficacy of the TNBS-induced colitis preclinical animal model by using in vitro cell cultures and the cell-specific epigenetic modulations of *NGF* gene regulation during inflammation.**
- **Confirm the crosstalk between hypermethylated DNA and histone modification in the regulation of *NGF* gene regulation by using the pharmacological inhibitors of histone methyltransferase (HMT) and acetyltransferase (HAT).**

## REFERENCES


- Hongjie Yuan, Shibin Du, Liping Chen, Xiaoqing Xu, Yufeng Wang and Fuhai Ji (2020) Hypomethylation of nerve growth factor (NGF) promotes binding of C/EBPα and contributes to inflammatory hyperalgesia in rats.
- Greeshma Ray, PhD and Michelle S. Longworth, PhD.(2019) Epigenetics, DNA Organization, and Inflammation Bowel Disease.
- F F di Mola, H Friess, Z W Zhu, A Koliopanos, T Bley, P Di Sebastiano, P Innocenti, A Zimmermann,M W Büchler (1999) Nerve growth factor and Trk high affinity receptor (TrkA) gene expression in inflammatory bowel disease.
- Michael J. Docherty, MD,corresponding author R. Carter W. Jones, III, MD, PhD, and Mark S. Wallace, MD. (2011) Managing Pain in Inflammatory Bowel Disease.
- Image is created using Biorender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