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Human Epididymis Protein 4 as a Potential Biomarker for Crohn's Disease-Associated Intestinal Fibrosis

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Article

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Abstract

Human epididymis protein 4 (HE4) plays a crucial role in promoting extracellular matrix (ECM) deposition by inhibiting matrix metalloproteinase (MMP) activity. Crohn's disease (CD) often leads to intestinal fibrosis and obstruction due to chronic inflammation. This study evaluated HE4 expression in serum and intestinal tissues from CD patients, and the effects of anti-HE4 antibody in a TNBS-induced mouse model of intestinal fibrosis. HE4 levels were significantly elevated in both serum and intestinal tissues of patients with stricturing CD, correlating with increased intestinal fibrosis and wall thickness. Inhibition of HE4 expression in mice reduced serum levels of IL-6, TNF-α, and intestinal expression of key fibrotic markers, including α-SMA, COL1A1, Fibronectin. These results suggest that HE4 is a potential biomarker for CD-associated intestinal fibrosis and a promising therapeutic target to mitigate fibrosis progression in CD.

Introduction

In recent years, the incidence of inflammatory bowel disease (IBD) in Asia has increased rapidly[1]. The pathogenesis of IBD is multifactorial, involving dietary patterns, genetic predisposition, immune dysregulation, environmental factors, and the intestinal microbiota[2]. Crohn’s disease (CD), a common subtype of IBD, is characterized pathologically by transmural inflammation that can extend through the muscularis propria to the serosal layer, often leading to complications such as intestinal stenosis and frequently requiring surgical resection of the affected intestinal segments[3]. Clinical observations indicate that intestinal obstruction is a frequent and severe complication, affecting approximately 39.9–48.2% of patients with a disease duration exceeding 20 years, many of whom eventually require surgical intervention or biologic therapy[4,5]. This fibrotic progression is currently attributed to an imbalance in the intestinal mucosal immune barrier, which triggers the release of various inflammatory cytokines that activate mesenchymal cells, leading to the excessive accumulation of extracellular matrix (ECM) components like fibrin[6] .

The human epididymis protein 4 (HE4) gene encodes a secreted glycoprotein with four whey acidic protein (WAP) domains that exhibits protease inhibitory activity. The WAP domains of HE4 are highly conserved across species and may play significant roles in antimicrobial and anti-inflammatory responses [7,8]. Functionally, HE4 has been shown to promote organ fibrosis by inhibiting the activity of matrix metalloproteinases (MMPs) through its WAP domain, thereby favoring the deposition of ECM [9,10]. Recent studies have reported HE4 expression in various fibrotic conditions, including renal fibrosis, hepatic fibrosis, idiopathic pulmonary fibrosis, and ischemic cardiomyopathy[11–14].

In the context of CD, the persistent elevation of pro-inflammatory cytokines such as TNF-α and IL-6 establishes a microenvironment that facilitates fibrosis[15]. Specifically, TNF-α can promote the production of IL-6 and directly stimulate the TGF-β1 signaling pathway, triggering the phosphorylation of Smad proteins (such as Smad3), inducing the expression of fibrosis-related genes and reducing matrix degradation[16]. However, its specific expression and role in CD-associated intestinal fibrosis remain unclear. Therefore, this study aims to evaluate whether HE4 could serve as a potential biomarker and therapeutic target for CD-associated intestinal fibrosis.

Materials and Methods

Patient Enrollment and Sample Collection

All patients with stenotic CD who underwent surgical treatment at our hospital were included in the experimental group (n = 20). Patients with intestinal obstruction who had normal tissue from both ends of the obstructed segment were included as controls(n = 8). The specimens were obtained from archived tissue samples in the Pathology Department of Hunan Provincial People's Hospital.

A cohort of 51 IBD patients (38 CD and 13 ulcerative colitis (UC)) admitted to Hunan Provincial People's Hospital between September 2021 and September 2022 was enrolled. A control group (CTR) included five healthy adults undergoing routine health examinations at the same hospital during the same period. All sample collection was performed following approval by the Medical Ethics Committee of Hunan Provincial People's Hospital (Ethical Approval No. 2022 − 191). All methods were performed in accordance with the relevant guidelines and regulations, including the Declaration of Helsinki. Written informed consent was obtained from all participants. On the first day of hospitalization, 3 mL of venous blood was collected from each patient into a coagulation tube, and serum was obtained after centrifugation. The 38 CD patients were further subdivided into four groups based on clinical diagnosis, imaging or endoscopic evidence of intestinal stricture, and the use of biologic agents (Infliximab): (1) stenotic CD (n = 16), (2) stenotic CD + biologic agents (n = 10), (3) non-stenotic CD (n = 6), and (4) non-stenotic CD + biologic agents (n = 6).

Clinical Data Collection

Clinical data were retrieved from the electronic medical record system of Hunan Provincial People's Hospital. Recorded parameters included patient age, gender, and laboratory values: serum albumin (ALB), hemoglobin (Hb), red blood cell count (RBC), absolute lymphocyte count (ALC), erythrocyte sedimentation rate (ESR), neutrophil

percentage (N%), and platelet count (PLT). The location of any stenotic intestinal segments was also recorded. Intestinal wall thickness was measured from imaging data by a specialist using the hospital’s medical imaging system. For CD patients, three measurements were taken from the most severely affected intestinal segment and averaged. For control subjects, the ascending, transverse, and descending colon wall thicknesses were measured and averaged[17].

TNBS-Induced Mouse Model

A TNBS-induced chronic intestinal fibrosis model was established using male BALB/c mice (8 weeks old, 18–20 g, n = 24; from Hunan SJA Laboratory Animal Co., Ltd). The mice were randomly assigned to four groups (n = 6 per group): (1) Saline, (2) TNBS, (3) TNBS + IgG, and (4) TNBS+HE4. Chronic colitis was induced by rectal administration of TNBS following a modified protocol[18]. After the fourth TNBS enema, mice in the TNBS + IgG and TNBS + HE4 groups received intraperitoneal injections of anti-IgG neutralizing antibody or anti-HE4 neutralizing antibody, respectively, while the Saline and TNBS groups received equivalent volumes of saline (5 mg/kg) twice weekly(Figure 1)[19]. Treatments continued for 3 weeks (six injections). Three days after the final injection, mice were fasted for 6 hours and then euthanized by CO2 asphyxiation followed by cervical dislocation to ensure death. Blood samples were collected via cardiac puncture, and tissues were harvested immediately and either snap-frozen in liquid nitrogen or fixed in 4% paraformaldehyde for subsequent analysis. All animal procedures were approved by the Medical Ethics Committee of Hunan Provincial People's Hospital (Ethical Approval No. 2022-17) and all methods were performed in accordance with relevant institutional guidelines and regulations, as well as the ARRIVE guidelines (v2.0) for reporting animal research.

Serum and Tissue Analysis

Serum HE4 levels in human samples and IL-6 and TNF-α, levels in mouse serum were measured by ELISA. Intestinal tissue samples from both humans and mice were fixed, embedded, and subjected to Masson’s trichrome staining to assess fibronectin deposition. Immunohistochemistry (IHC) was used to detect expression of HE4, α-SMA, and collagen. Fresh mouse colon tissue samples were homogenized and protein extracts were analyzed by Western blot to quantify α-SMA, COL3A1, COL1A1, fibronectin, TGF-β1, and phosphorylated Smad3 (P-Smad3/Smad3) levels.

Statistical analysis

The data are expressed as mean ± standard deviation. Spearman's rank correlation analysis was used to evaluate the correlation. Grayscale values from Western blot analyses

and the percentage of positive cells from immunohistochemistry were quantified using ImageJ software. In the Western blot analysis, GAPDH served as the internal reference protein. Each sample's grayscale value was measured in triplicate, and the mean of these measurements was utilized as the final value. The relative expression levels of the target protein were determined by normalizing its grayscale value to that of GAPDH. All graphical representations and statistical analyses were conducted using GraphPad Prism version 8.0. Comparisons of continuous variables between two groups were analyzed using Student's t-test, while categorical variables were compared using the Chi-square test, with statistical significance set at a p-value of less than 0.05. Significance levels are indicated as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

Results

HE4 Expression Is Elevated in IBD Patient Serum

Serum HE4 levels were measured by ELISA in the control, CD, and UC groups. As shown in Fig. 2, serum HE4 levels were significantly higher in the CD group (6123 ± 2292.2 pg/mL) and UC group (7482.4 ± 2861.6 pg/mL) than in the control group (3817.4 ± 771.0 pg/mL) ($P < 0.05$). The increase in the UC group was more significant ($P < 0.01$), whereas the difference between the CD and UC groups was not statistically significant ($P > 0.05$) .

Biologic Agents Reduce Serum HE4 Levels in Stenotic CD Patients

We compared serum HE4 levels among the CD patient subgroups (Fig. 3). Stenotic CD patients not treated with biologic agents had higher serum HE4 levels (7397.3 ± 2363.5 pg/mL) than the control group ($P < 0.01$) and also higher than stenotic CD patients treated with biologics (4630.8 ± 1460.7 pg/mL, $P < 0.05$). No significant differences were observed between the stenotic CD patients treated with biologics, the non-stenotic CD patients (with or without biologics), and the control group ($P > 0.05$ for all comparisons).

Correlation Between Serum HE4 Levels and Clinical Indicators

We analyzed the correlation between serum HE4 levels and clinical parameters (Fig. 4). Serum HE4 levels were positively correlated with the thickness of the most stenotic intestinal segment ($R = 0.608$, $P < 0.001$), ESR ($R = 0.478$, $P < 0.001$), percentage of neutrophil ($R = 0.441$, $P < 0.001$), platelet count (PLT) ($R = 0.583$, $P < 0.001$), and patient age ($R = 0.439$, $P = 0.001$). HE4 levels were negatively correlated with serum albumin (ALB) (R=-0.514, $P < 0.001$), hemoglobin (R=-0.422, $P = 0.002$), RBC count (R=-0.451, P <

0.001), and absolute lymphocyte count (R=-0.364, $P = 0.009$). No significant correlation was found between HE4 levels and patient gender or the location of stenosis (ileum, colon, or ileocolon) ($P > 0.05$ for all).

Fibronectin and HE4 Expression in Stenotic CD Intestinal Tissue

Masson's trichrome staining and IHC were performed on intestinal tissue specimens (Fig. 5). Compared to controls, stenotic CD tissues showed extensive fibronectin deposition in the submucosa, rupture of the muscularis mucosa, and destruction of normal tissue architecture. HE4 expression was increased in the mucosal and submucosal layers. Chi-square test analysis revealed that HE4 expression was significantly higher in stenotic CD tissues compared to the CTR group ($p < 0.05$).

Macroscopic Appearance of Mouse Colon Tissue

The colon length and gross appearance of mice in each group were evaluated. The Saline and TNBS + HE4 groups had normal colon length and uniform coloration, with consistent wall thickness and no necrosis, distortion, or stenosis. In contrast, the TNBS and TNBS + IgG groups exhibited colons with localized edema and lumen stenosis, and the colonic walls appeared rigid, twisted, and deformed(Figure 6). However, differences in colon length and weight between groups were not statistically significant ($P > 0.05$).

Inhibition of HE4 Expression Reduces Inflammatory Cytokine Release

Serum IL-6 and TNF-α levels were measured by ELISA. IL-6 and TNF-α levels were significantly higher in the TNBS group than in the Saline group ($P < 0.05$). In the TNBS + IgG group, IL-6 levels were not significantly different from c Salines, but TNF-α levels remained elevated ($P < 0.01$). Both IL-6 and TNF-α levels were significantly lower in the TNBS + HE4 group compared to the TNBS and TNBS + IgG groups ($P < 0.05$), and were not significantly different from the Saline group(Figure 7). These results indicate that HE4 inhibition attenuated the release of inflammatory cytokines.

Inhibition of HE4 Expression Ameliorates Chronic Intestinal Fibrosis in Mice

Masson's trichrome and IHC staining (Fig. 8) revealed that fibronectin deposition was significantly higher in the submucosa and muscularis propria of the TNBS and TNBS + IgG groups compared to the Saline and TNBS+HE4 groups, with disorganized tissue structure. Chi-square test analysis showed that the expression levels of α-SMA and collagen I were

significantly increased in the TNBS and TNBS + IgG groups compared with the Saline and TNBS + HE4 groups ($P < 0.0001$). In these groups, α-SMA and collagen I expression were also significantly increased in the mucosal, submucosal, and muscular layers. By contrast, the TNBS+HE4 group showed normal colon tissue structure and low fibronectin expression in the submucosa, comparable to Salines. In this group, α-SMA and collagen I levels in all layers did not differ significantly from the Saline group.

Western blot analysis (Fig. 9) confirmed that protein levels of α-SMA, COL3A1, COL1A1, fibronectin, TGF-β1, and phosphorylated Smad3 (P-Smad3/Smad3) were significantly higher in the TNBS group than in Salines, indicating successful induction of fibrosis. In the TNBS+HE4 group, levels of fibronectin, COL3A1, COL1A1, P-Smad3/Smad3, α-SMA, and TGF-β1 were significantly lower than in the TNBS + IgG group ($P < 0.05$ for each), demonstrating that anti-HE4 treatment alleviated intestinal fibrosis by suppressing inflammatory cytokine production and extracellular matrix deposition.

Discussion

Intestinal fibrosis is a complex and dynamic process. It is currently believed that, on the basis of genetic susceptibility [20], the imbalance of the intestinal mucosal immune barrier triggers the release of various inflammatory cytokines, which further activate intestinal mesenchymal cells, leading to excessive deposition of ECM components such as fibrin, which in turn induces fibrosis and subsequently causes segmental intestinal stricture or even obstruction [6]. Intestinal obstruction is one of the most common complications of CD, occurring in 39.9–48.2% of patients with a disease duration of over 20 years, most of whom require surgical intervention or biologic agents[4,5].

HE4, a WAP-domain glycoprotein, can inhibit matrix metalloproteinases (MMPs) via its WAP domain, leading to extracellular matrix (ECM) deposition and subsequent organ fibrosis[9,10]. While HE4 expression is typically confined to the epididymis and selected normal tissues with negligible levels in the normal colon, evidence from Kemal et al. demonstrates significant HE4 overexpression within colonic epithelial cells during malignancy, notably in advanced-stage (III-IV) colorectal cancer [21,22]. Such a shift in expression patterns indicates that the transcription of HE4 in the intestinal epithelium is inducible and can be triggered by specific pathophysiological stimuli, reinforcing its role as a responsive mediator in intestinal pathology. Previous studies have implicated HE4 in cystic fibrosis, renal fibrosis, and liver fibrosis[9,23,24]. Therefore, we explored whether HE4 could serve as a biomarker for CD-associated intestinal fibrosis.

Clinical evaluation of CD often relies on symptoms, laboratory tests, and imaging, where severe anemia, hypoalbuminemia, elevated neutrophil percentage, and ESR typically indicate active disease[25]. Previous studies have shown that combining HE4 with other

biomarkers improves diagnostic accuracy for ovarian cancer[26]. In our analysis, serum HE4 levels correlated positively with intestinal wall thickness, ESR, neutrophil percentage, platelet count, and age, and negatively with albumin, hemoglobin, RBC count, and lymphocyte count. The particularly strong correlation between HE4 and wall thickness suggests that HE4 may be useful for early prediction of CD-associated intestinal fibrosis.

In this study, we found that the stenotic CD patients without biologic therapy (specifically Infliximab) had significantly higher serum HE4 levels than the control group. In contrast, there were no significant differences between non-stenotic CD patients (with or without biologics) and controls. Moreover, serum HE4 levels in untreated stenotic CD patients were significantly higher than in biologic-treated stenotic CD patients, indicating that HE4 expression is elevated in CD and shows specific expression in stenotic CD, and that biologic therapy may reduce serum HE4 levels. Our findings align with current clinical practices where biologic agents targeting TNF-α, IL-6, and leukocyte adhesion molecules improve CD treatment outcomes by blocking key inflammatory pathways [27,28]. Infliximab, as a TNF-α inhibitor, plays a critical role in autoimmune diseases by suppressing inflammation. TNF-α has been confirmed to induce the upregulation of IL-6, a key pro-inflammatory cytokine involved in acute phase responses and chronic inflammation [29]. Considering that excessive IL-6 production has been implicated in hepatic, pulmonary, and renal fibrosis [30,31], it is plausible that inflammatory factors contribute to HE4 expression and drive the progression of CD-associated intestinal fibrosis.

Thus, we established a TNBS-induced chronic intestinal fibrosis model in mice and demonstrated that inhibition of HE4 expression via neutralizing antibodies ameliorates fibrosis. The TNBS group showed shortened, rigid, and narrowed colons, along with elevated serum IL-6 and TNF-α levels and increased collagen in colon tissue, indicating successful fibrosis modeling [18]. In contrast, the TNBS + HE4 group displayed reduced serum IL-6 and TNF-α levels compared to the TNBS + IgG group, as well as decreased expression of TGF-β1 and downstream P-Smad3/Smad3 in colon tissue, alongside reduced expression of fibrosis-associated proteins including α-SMA, COL3A1, COL1A1, and fibronectin. These results suggest that HE4 contributes to intestinal fibrosis via inflammation-mediated pathways and that HE4 inhibition mitigates fibrosis by suppressing inflammatory cytokine production and ECM deposition.

The imbalance of the intestinal mucosal immune barrier in CD leads to the release of various inflammatory cytokines. Pro-inflammatory cytokines, including IL-6 and TNF-α, are persistently elevated in CD and contribute to sustained inflammatory responses and repeated cycles of intestinal injury and repair, thereby promoting a profibrotic microenvironment [15]. Accumulating evidence indicates that increased levels of IL-6 and TNF-α are associated with enhanced expression of TGF-β1, suggesting that these

inflammatory cytokines may act as upstream modulators of fibrotic responses [32]. As a core cytokine in CD, TNF-α further triggers inflammatory responses and immune cell activation; it not only promotes IL-6 production but also directly activates the TGF-β1 signaling pathway. Both TNF-α and IL-6 can enhance the expression of profibrotic factors, including TGF-β1. Concurrently, IL-6, as another key inflammatory cytokine, can further stimulate TGF-β1 secretion to amplify the fibrotic process and enhance TGF-β1 activity. TGF-β1 is widely recognized as a central profibrotic mediator in CD; once activated, the TGF-β1 signaling pathway triggers a cascade of intracellular events, including the phosphorylation of Smad proteins (specifically Smad2 and Smad3). These phosphorylated Smad proteins subsequently translocate to the nucleus to induce the expression of fibrosis-related genes, which is closely associated with fibroblast activation, ECM deposition, and reduced matrix degradation [16]. Recent studies have identified HE4 as an important regulator of fibrotic remodeling through its inhibitory effects on matrix metalloproteinase activity, thereby favoring ECM accumulation [9]. In the context of chronic intestinal inflammation, increased TGF-β1 expression may contribute to HE4 upregulation, which in turn shifts intestinal tissue repair toward fibrosis [33]. Collectively, these findings suggest that elevated IL-6 and TNF-α levels, enhanced TGF-β1 signaling, and HE4-mediated impairment of ECM degradation are closely associated with the development of intestinal fibrosis in CD, highlighting HE4 as a potential biomarker and therapeutic target for fibrotic complications. The interaction between TNF-α, IL-6, HE4, and TGF-β1 amplifies the fibrotic process in CD, ultimately leading to the formation of strictures and intestinal obstruction.

Conclusions

This study demonstrated that HE4 is specifically upregulated in CD-associated intestinal fibrosis and correlates with disease severity markers. Preliminary mouse model experiments support a role for HE4 in promoting fibrosis through inflammatory pathways. Further large-scale clinical studies are required to validate whether HE4 can serve as an early biomarker for CD-associated intestinal fibrosis and potential therapeutic target.

Declarations

Author contributions

L.Z.H., X.X.P. and conceived and designed the experiments; L.M.Z, Z.J.F. and X.L.Z. performed the experiments; and Z.Y.Y. contributed to the statistical data analysis; Z.M.H., L.S.P. and P.Y. wrote the manuscript; L.X.Q., W.Y.Z. and W.M.H. corrected the manuscript. All authors have read and approved the manuscript for publication.

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Data availability

The data sets generated and analyzed during this study are available from the corresponding author on reasonable request.

Ethical Approval and Consent to participate

Subjects were recruited from Hunan Provincial People's Hospital. All participants provided informed consent, and no prison populations or their organs were used. This study was performed in line with the principles of the Declaration of Helsinki. And the study is reported in accordance with ARRIVE guidelines (v2.0). Approval was granted by the Medical Ethics Committee of Hunan Provincial People's Hospital (Ethical Approval No. 2022-191) (Ethical Approval No. 2022-17).

Declaration of Competing Interest

All the authors declare no conflict of interest

References

1. Ng, S. C. et al. Incidence and phenotype of inflammatory bowel disease based on results from the Asia-pacific Crohn's and colitis epidemiology study. *Gastroenterology* **145**, 158–165e152. 10.1053/j.gastro.2013.04.007 (2013).
2. Butera, A. et al. CD147 Targeting by AC-73 Induces Autophagy and Reduces Intestinal Fibrosis Associated with TNBS Chronic Colitis. *J. Crohn's colitis*. **16**, 1751–1761. 10.1093/ecco-jcc/jjac084 (2022).
3. Latella, G. et al. Results of the 4th scientific workshop of the ECCO (I): pathophysiology of intestinal fibrosis in IBD. *J. Crohn's colitis*. **8**, 1147–1165. 10.1016/j.crohns.2014.03.008 (2014).
4. Cleynen, I. et al. Genetic factors conferring an increased susceptibility to develop Crohn's disease also influence disease phenotype: results from the IBDchip European Project. *Gut* **62**, 1556–1565. 10.1136/gutjnl-2011-300777 (2013).
5. Ng, S. C. et al. Early Course of Inflammatory Bowel Disease in a Population-Based Inception Cohort Study From 8 Countries in Asia and Australia. *Gastroenterology* **150**, 86–95. 10.1053/j.gastro.2015.09.005 (2016). e83; quiz e13-84.
6. Pariente, B. et al. Development of the Lémann index to assess digestive tract damage in patients with Crohn's disease. *Gastroenterology* **148**, 52–63e53. 10.1053/j.gastro.2014.09.015 (2015).

7. Galgano, M. T., Hampton, G. M. & Frierson, H. F. Jr. Comprehensive analysis of HE4 expression in normal and malignant human tissues. *Mod. pathology: official J. United States Can. Acad. Pathol. Inc*. **19**, 847–853. 10.1038/modpathol.3800612 (2006).
8. Clauss, A., Lilja, H. & Lundwall, A. The evolution of a genetic locus encoding small serine proteinase inhibitors. *Biochem. Biophys. Res. Commun.* **333**, 383–389. 10.1016/j.bbrc.2005.05.125 (2005).
9. LeBleu, V. S. et al. Identification of human epididymis protein-4 as a fibroblast-derived mediator of fibrosis. *Nat. Med.* **19**, 227–231. 10.1038/nm.2989 (2013).
10. Bingle, L., Singleton, V. & Bingle, C. D. The putative ovarian tumour marker gene HE4 (WFDC2), is expressed in normal tissues and undergoes complex alternative splicing to yield multiple protein isoforms. *Oncogene* **21**, 2768–2773. 10.1038/sj.onc.1205363 (2002).
11. Hou, Y. et al. Clinical significance of serum human epididymis protein 4 in liver fibrosis: An experimental study. *Medicine* **99**, e23428. 10.1097/md.0000000000023428 (2020).
12. Piek, A. et al. HE4 Serum Levels Are Associated with Heart Failure Severity in Patients With Chronic Heart Failure. *J. Card. Fail.* **23**, 12–19. 10.1016/j.cardfail.2016.05.002 (2017).
13. Raghu, G. et al. Idiopathic Pulmonary Fibrosis: Prospective, Case-Controlled Study of Natural History and Circulating Biomarkers. *Chest* **154**, 1359–1370. 10.1016/j.chest.2018.08.1083 (2018).
14. Tang, Y. et al. Human epididymis protein 4: a novel predictor of ischemic cardiomyopathy. *BMC Cardiovasc. Disord.* **21** 10.1186/s12872-021-02319-5 (2021).
15. Neurath, M. F. Strategies for targeting cytokines in inflammatory bowel disease. *Nat. Rev. Immunol.* **24**, 559–576. 10.1038/s41577-024-01008-6 (2024).
16. Qiu, D. et al. NQO1 alleviates renal fibrosis by inhibiting the TLR4/NF-κB and TGF-β/Smad signaling pathways in diabetic nephropathy. *Cell. Signal.* **108**, 110712. 10.1016/j.cellsig.2023.110712 (2023).
17. Meng, J. et al. Intestinal fibrosis classification in patients with Crohn's disease using CT enterography-based deep learning: comparisons with radiomics and radiologists. *Eur. Radiol.* **32**, 8692–8705. 10.1007/s00330-022-08842-z (2022).
18. Wirtz, S. et al. Chemically induced mouse models of acute and chronic intestinal inflammation. *Nat. Protoc.* **12**, 1295–1309. 10.1038/nprot.2017.044 (2017).
19. Scheibe, K. et al. Inhibiting Interleukin 36 Receptor Signaling Reduces Fibrosis in Mice With Chronic Intestinal Inflammation. *Gastroenterology* **156**, 1082–1097e1011. 10.1053/j.gastro.2018.11.029 (2019).
20. Bettenworth, D. & Rieder, F. Pathogenesis of Intestinal Fibrosis in Inflammatory Bowel Disease and Perspectives for Therapeutic Implication. *Dig. Dis. (Basel Switzerland)*.

35, 25–31. 10.1159/000449079 (2017).

21. Kirchhoff, C., Habben, I., Ivell, R. & Krull, N. A major human epididymis-specific cDNA encodes a protein with sequence homology to extracellular proteinase inhibitors. *Biol. Reprod.* **45**, 350–357. 10.1095/biolreprod45.2.350 (1991).
22. Kemal, Y. N. et al. Serum human epididymis protein 4 levels in colorectal cancer patients. *Mol. Clin. Oncol.* **7**, 481–485. 10.3892/mco.2017.1332 (2017).
23. Chen, J. et al. Elevated Serum Human Epididymis Protein 4 Is Associated With Disease Activity and Systemic Involvements in Primary Sjögren's Syndrome. *Front. Immunol.* **12**, 670642. 10.3389/fimmu.2021.670642 (2021).
24. Bingle, L. et al. WFDC2 (HE4): a potential role in the innate immunity of the oral cavity and respiratory tract and the development of adenocarcinomas of the lung. *Respir. Res.* **7**, 61. 10.1186/1465-9921-7-61 (2006).
25. Kessel, C. et al. Serum biomarkers confirming stable remission in inflammatory bowel disease. *Sci. Rep.* **11**, 6690. 10.1038/s41598-021-86251-w (2021).
26. Hameed, S., Abdulqader Jasim, H. & Sharief, M. Effect of Serum Level of Human Epididymis Protein 4 and Interleukin-6 as Biomarkers in Patients with Adnexal Mass. *Arch. Razi Inst.* **77**, 1659–1671. 10.22092/ari.2022.358329.2194 (2022).
27. Triantafillidis, J. K., Zografos, C. G., Konstadoulakis, M. M. & Papalois, A. E. Combination treatment of inflammatory bowel disease: Present status and future perspectives. *World J. Gastroenterol.* **30**, 2068–2080. 10.3748/wjg.v30.i15.2068 (2024).
28. Nakase, H. et al. Evidence-based clinical practice guidelines for inflammatory bowel disease 2020. *J. Gastroenterol.* **56**, 489–526. 10.1007/s00535-021-01784-1 (2021).
29. Wang, Y. et al. Metformin Ameliorates Chronic Colitis-Related Intestinal Fibrosis via Inhibiting TGF-β1/Smad3 Signaling. *Front. Pharmacol.* **13** 10.3389/fphar.2022.887497 (2022).
30. Zhang, K. et al. Targeting GPR65 alleviates hepatic inflammation and fibrosis by suppressing the JNK and NF-κB pathways. *Military Med. Res.* **10** 10.1186/s40779-023-00494-4 (2023).
31. Wang, Y. et al. Xuanfei Baidu Decoction protects against macrophages induced inflammation and pulmonary fibrosis via inhibiting IL-6/STAT3 signaling pathway. *J. Ethnopharmacol.* **283**, 114701. 10.1016/j.jep.2021.114701 (2022).
32. Wynn, T. A. Cellular and molecular mechanisms of fibrosis. *J. Pathol.* **214**, 199–210. 10.1002/path.2277 (2008).
33. Zhang, L. et al. Hypoxia-induced HE4 in tubular epithelial cells promotes extracellular matrix accumulation and renal fibrosis via NF-κB. *FASEB journal: official publication Federation Am. Soc. Experimental Biology*. **34**, 2554–2567. 10.1096/fj.201901950R (2020).

Figures

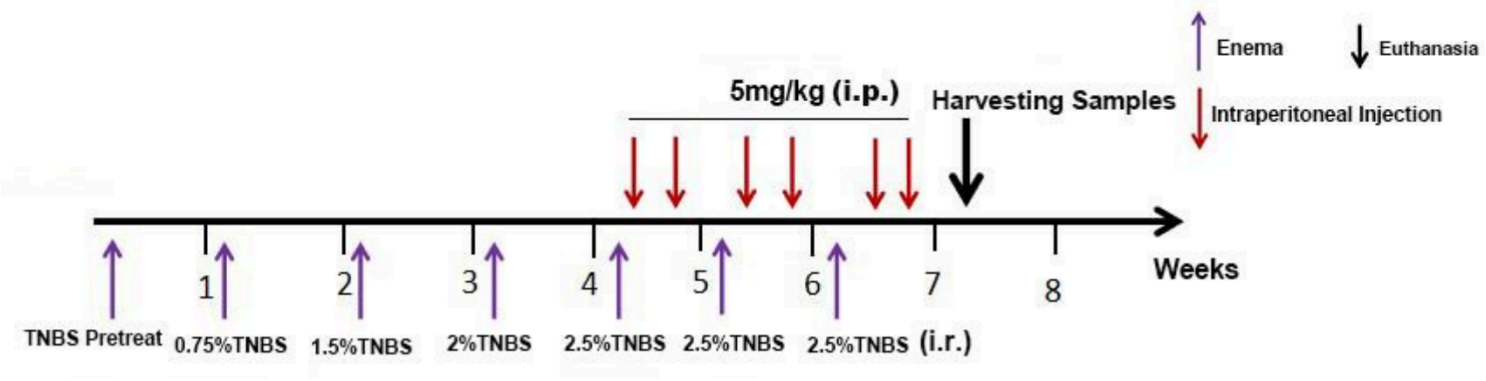


Figure 1

Schematic of the TNBS-induced intestinal fibrosis mouse model.
An experimental timeline depicting the induction of intestinal fibrosis in male C57BL/6 mice (approximately 8 weeks old). Prior to intrarectal administration, mice were presensitized by epicutaneous application of TNBS on a shaved area of the dorsal skin to assess potential allergic reactions. Subsequently, intestinal fibrosis was induced via intrarectal (*i.r.*) administration of TNBS at varying concentrations and time points, combined with intraperitoneal (*i.p.*) injections (5 mg/kg). Colonic tissue samples were harvested from the mice at 7 weeks post-modeling.*Abbreviations: i.r., intrarectal; i.p., intraperitoneal.*

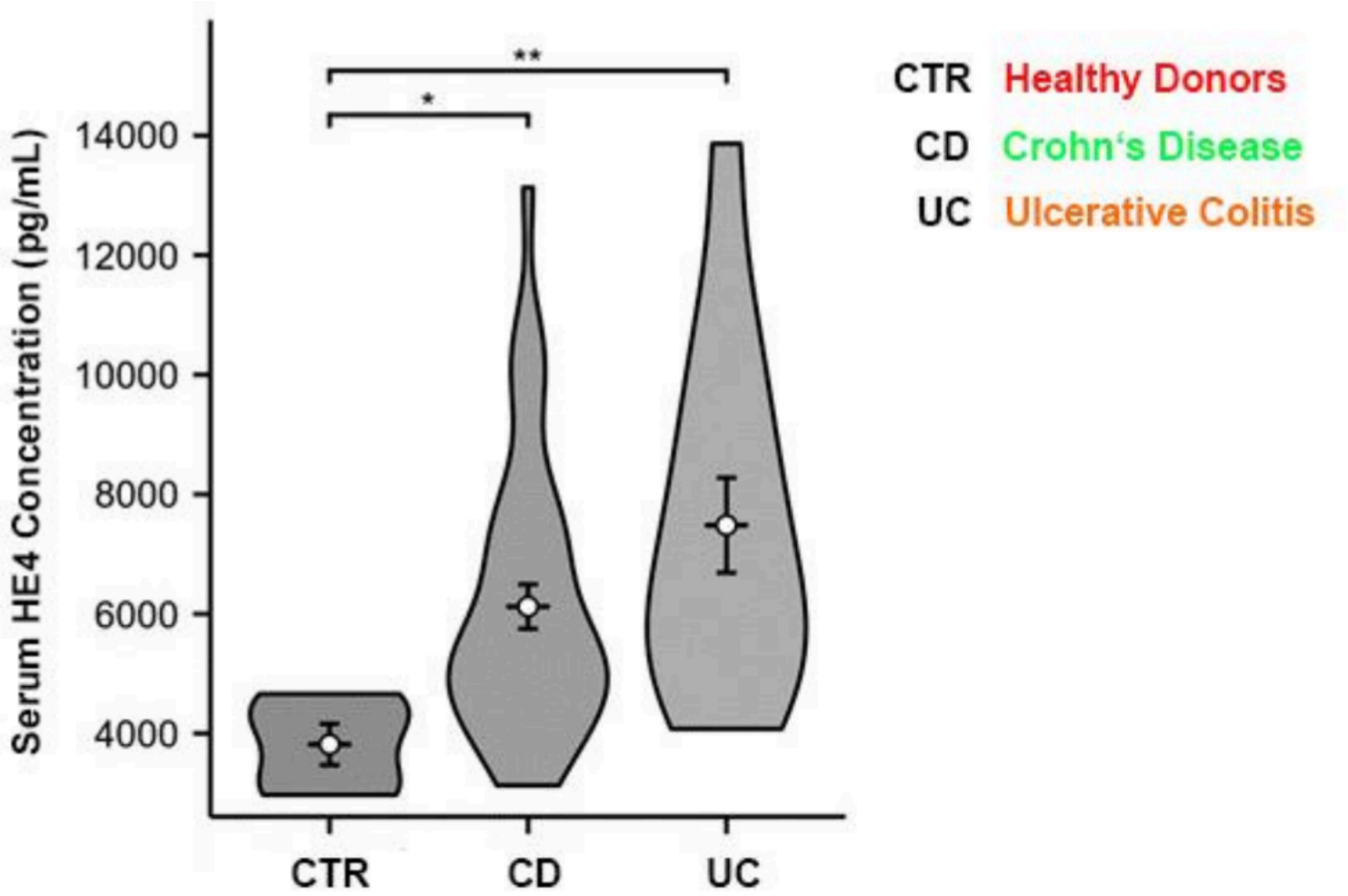


Figure 2

Serum HE4 concentration in human subjects measured by ELISA.
Serum levels of HE4 were quantified by ELISA in healthy controls (CTR), patients with Crohn's disease (CD), and patients with ulcerative colitis (UC). Blood samples were collected, centrifuged, and the supernatant was analyzed. Experiments were performed in at least triplicate. Data are presented as mean ± SD. Statistical significance was determined by Student's t-test (*$p < 0.05$, **$p < 0.01$*).

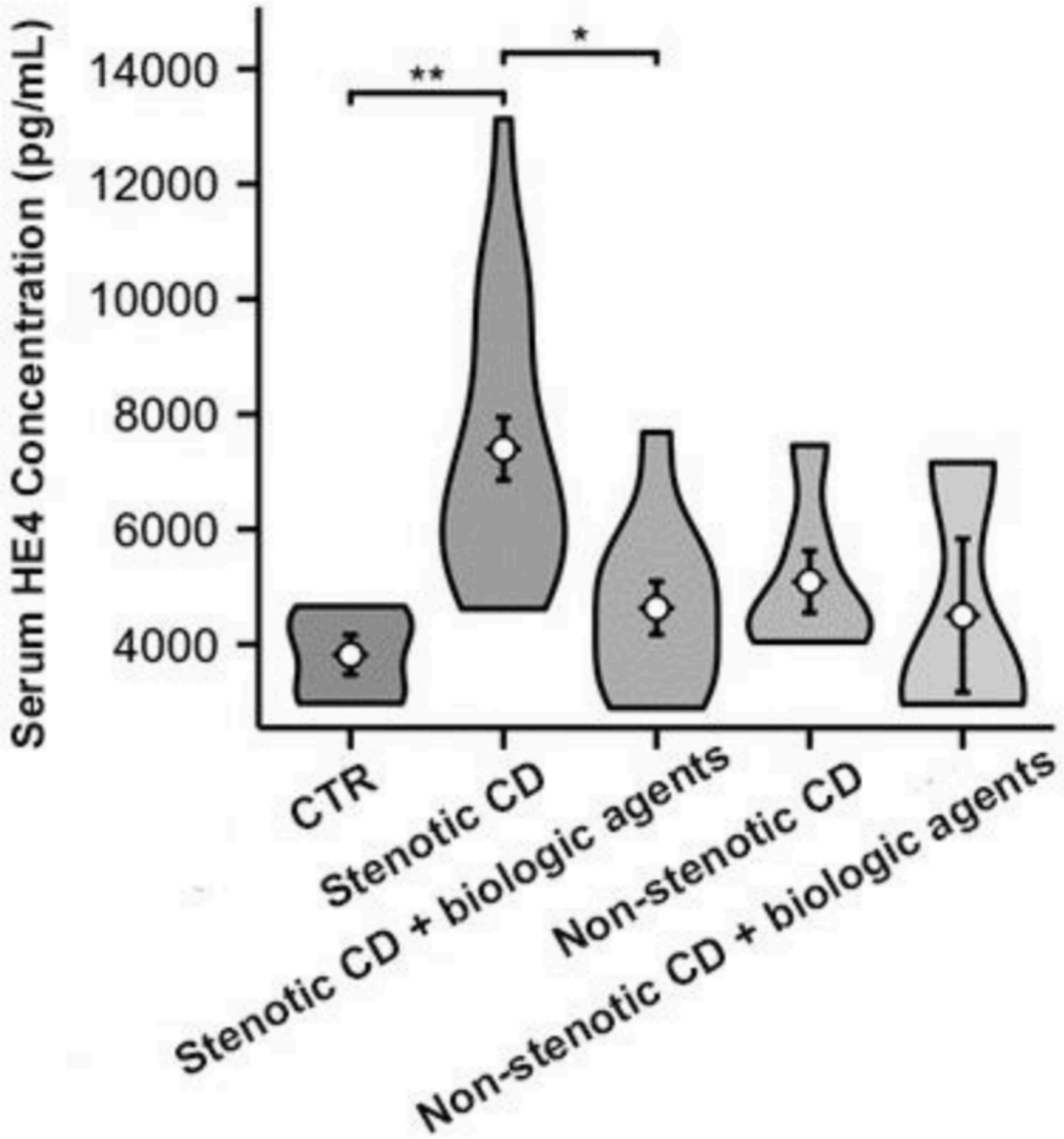


Figure 3

Serum HE4 levels in CD patient subgroups with or without biologic therapy.
Serum HE4 concentrations were compared across five groups: healthy controls (CTR), stenotic CD , stenotic CD + biologic agents , non-stenotic CD, non-stenotic CD + biologic agents .Data are presented as mean ± SD. Differences were analyzed by Student's t-test (*$p < 0.05$, **$p < 0.01$*).

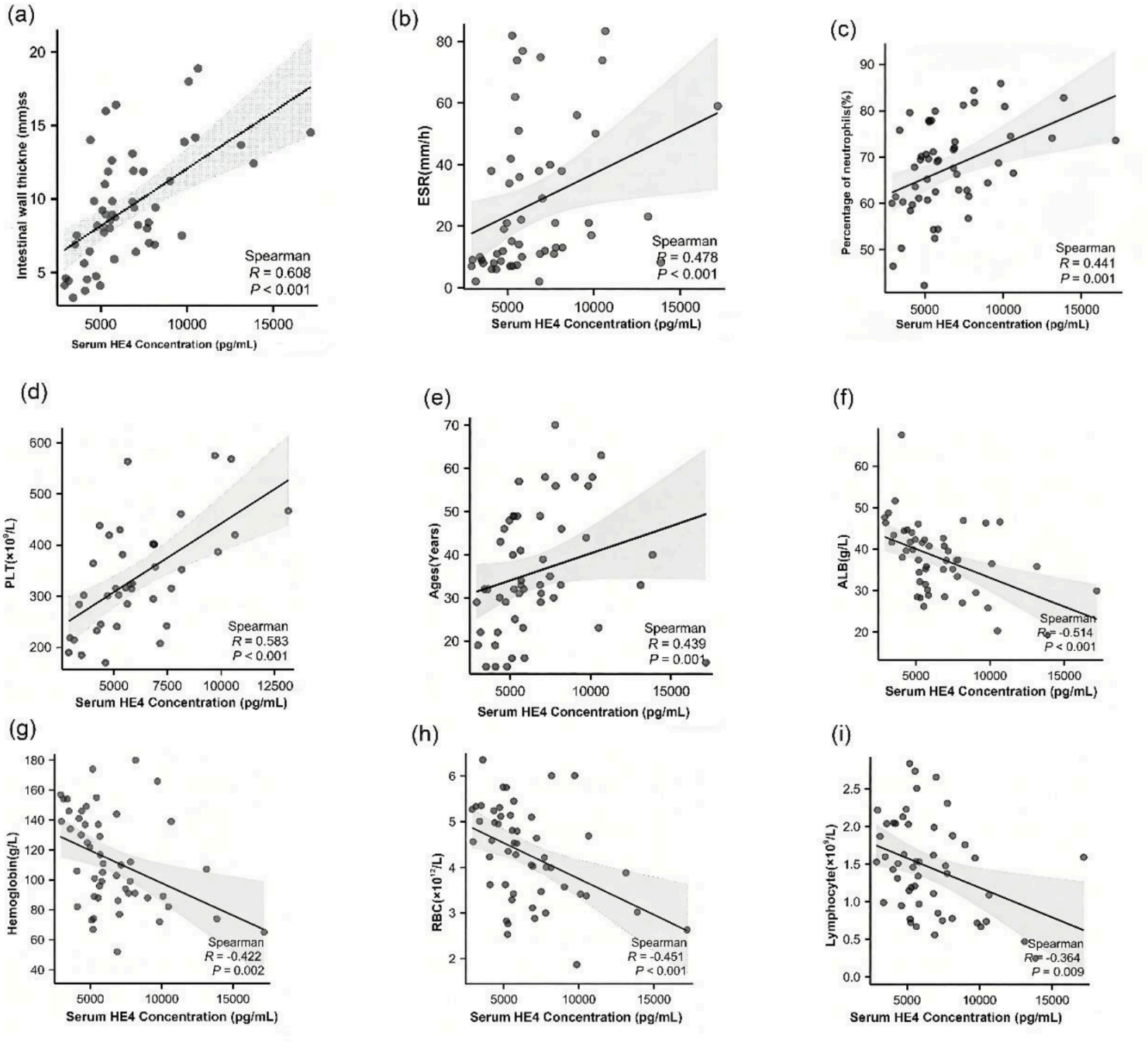


Figure 4

Correlation Between Serum HE4 Levels and Clinical Indicators

Spearman's rank correlation analysis was performed to assess the association of serum HE4 levels with (a) intestinal wall thickness and (b–i) various clinical indicators. The correlation coefficient (R) ranges from −1 to +1, where values closer to ±1 indicate stronger monotonic relationships.

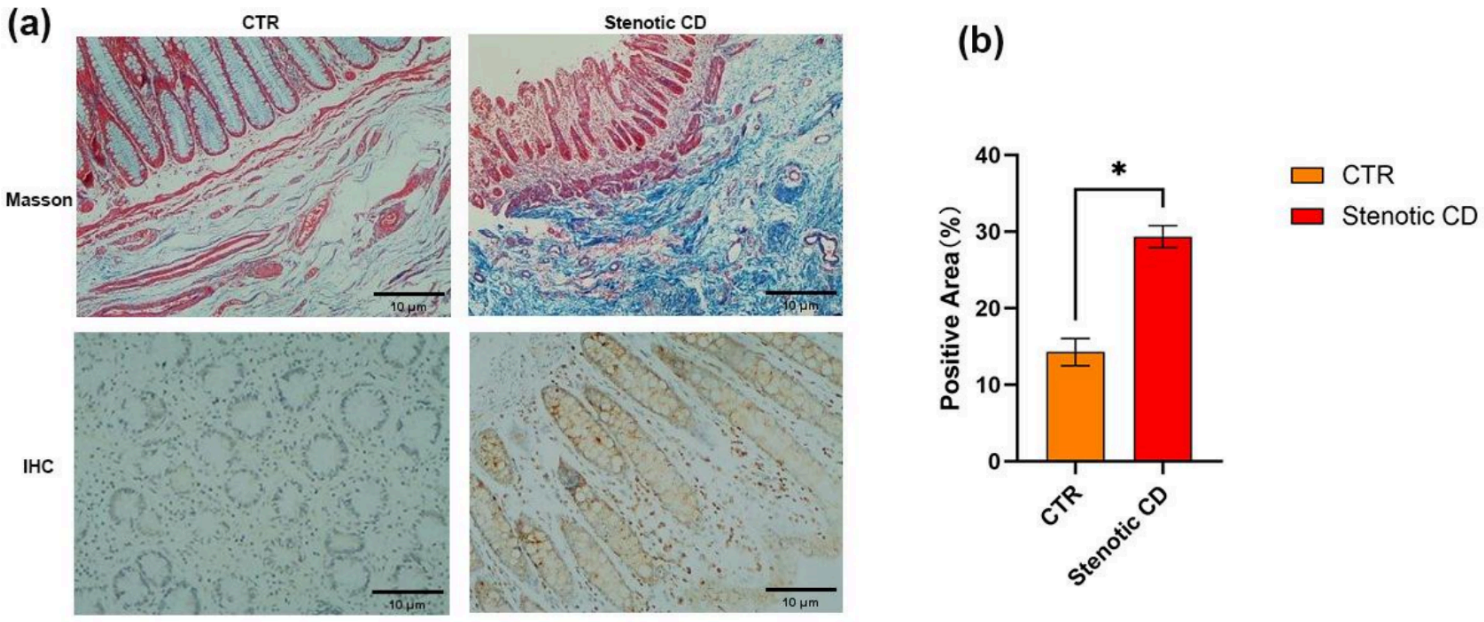


Figure 5

Histopathological evaluation of human intestinal tissues.
(a) Representative images of Masson's trichrome staining and immunohistochemical (IHC) staining for HE4 in human colon sections. (b) Quantitative analysis of IHC staining positivity was performed using ImageJ. Differences in positive rates between groups were compared by Chi-square test ($p < 0.05$).



Figure 6

Macroscopic Appearance of Mouse Colon Tissue

Colon tissues were collected after euthanasia, rinsed with cold physiological saline, and photographed. The TNBS and TNBS+IgG groups exhibited colons with localized edema and lumen stenosis, and the colonic walls appeared rigid, twisted, and deformed.

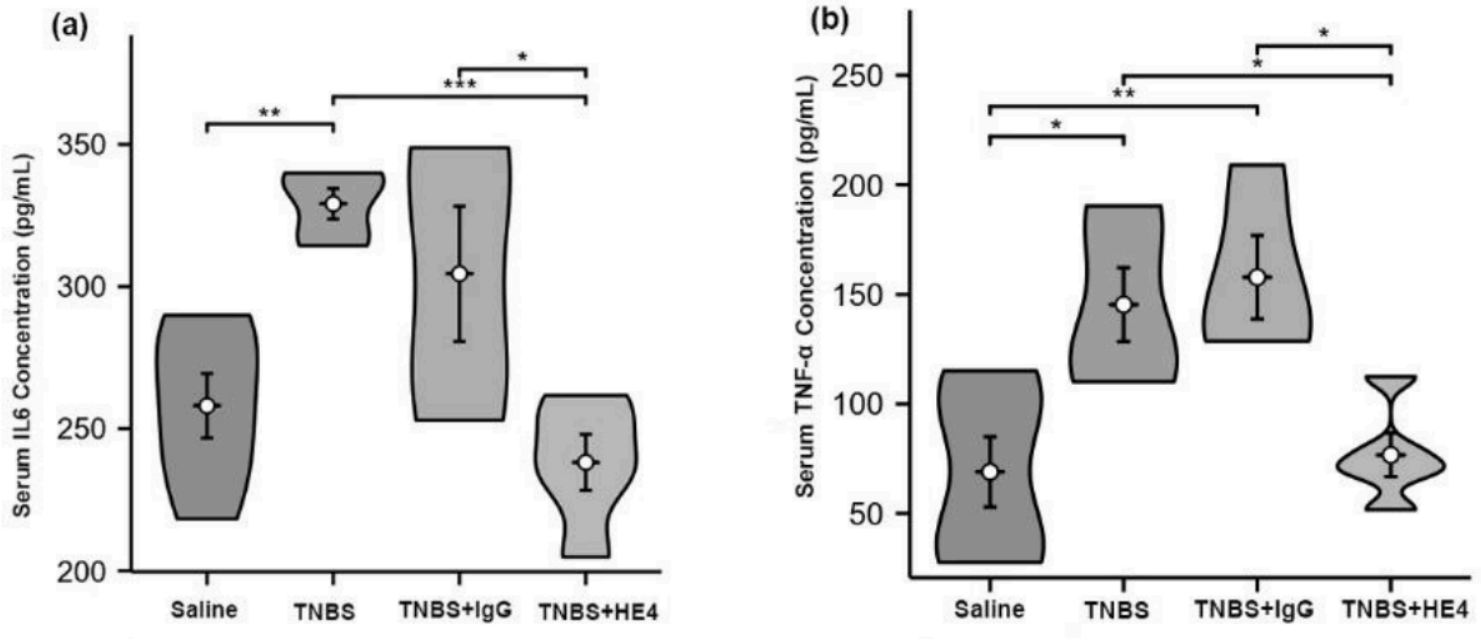


Figure 7

Serum cytokine levels in mice measured by ELISA.
Concentrations of (a) IL-6 and (b) TNF-α in mouse serum were determined by ELISA. Data are presented as mean ± SD. Statistical comparisons between groups were performed using Student's t-test (*p < 0.05, **p < 0.01, ***p < 0.001*).

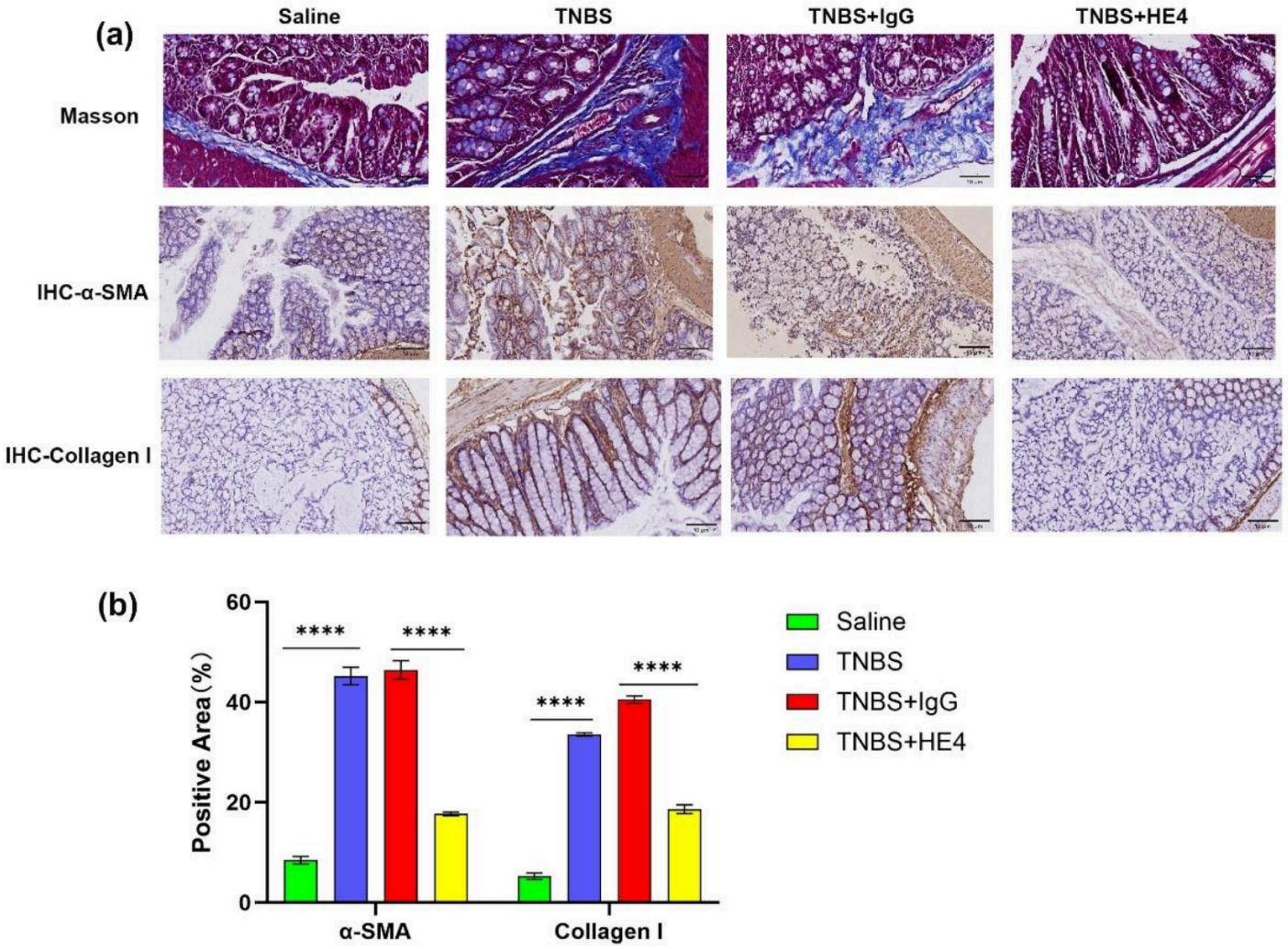


Figure 8. Histopathological staining of mouse colon tissues. (a) Representative images of Masson's trichrome staining and immunohistochemistry (IHC) for relevant markers in different intervention groups. (b) IHC positivity was quantified using ImageJ across at least three different regions per sample. Statistical analysis was performed using Chi-square test (***$p < 0.0001$).

Figure 8

See image above for figure legend

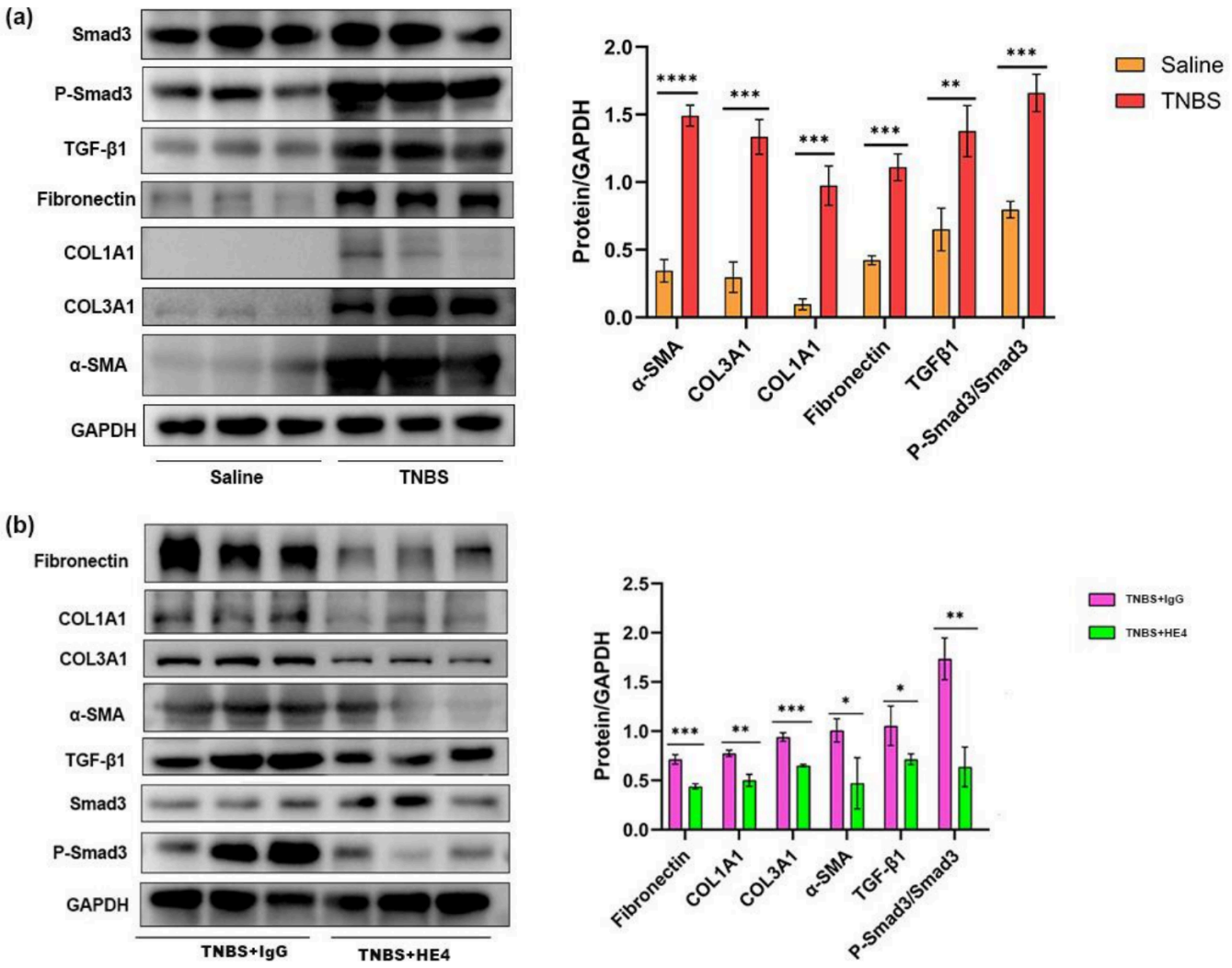


Figure 9. Western blot analysis of mouse colon tissues from the TNBS model. Protein expression profiles were compared between (a) TNBS vs. Saline groups and (b) TNBS+HE4 vs. TNBS+IgG groups. Band intensity was quantified using ImageJ, with at least three independent measurements averaged per sample. GAPDH was used as a loading control, and relative expression was calculated as the ratio of target protein to GAPDH. Statistical significance was determined by Student's t-test (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001*).

Figure 9

See image above for figure legend

Supplementary Files

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- OriginalWBImages.zip