Tripterygium wilfordii polycoride regulates S100A8/TLR4 pathway in ulcerative colitis

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Abstract:

Ethnopharmacological relevance:

Tripterygium wilfordii Hook F. (TwHF) , a member of the Euonymus family, is a traditional Chinese herbal medicine in China. It possesses various beneficial properties including anti-inflammatory, immunosuppressive, anti-tumor, and anti-viral effects. It is commonly utilized in the treatment of glomerulonephritis and rheumatoid arthritis. *Tripterygium wilfordii* polycoride (TWP), a fat-soluble mixture extracted and refined from the root of *Tripterygium wilfordii Hook F*., is the first Chinese herbal medicine in my country to exhibit anti-inflammatory and immune-regulating properties. It is popularly referred to as the 'Chinese herbal hormone' and warrants further research.

Aim of the study: The aim of this study is to identify the core genes and investigate the mechanisms underlying the therapeutic effects of TWP on UC.

Materials and methods: This study is divided into three parts. Firstly, we conducted a transcriptome analysis of UC rat colon to identify core genes related to inflammation and TWP treatment. Secondly, we verified the expression and function of core genes in vivo and in vitro experiments. Finally, we evaluated the anti-inflammatory efficacy and mechanism of TWP.

Results: The study found that S100A8 expression increased by 143 times in model group, but decreased by 148 times after TWP treatment. The study revealed that inflammation led to increase the expression of S100A8, TLR4 signaling pathways, and inflammatory factors in vitro, while anti-inflammatory factors decreased. RhS100A8 increased the expression of TLR4 pathway and inflammatory factors, while inhibiting the activity of TLR4 with TAK-242 decreased the expression of downstream pathways. The study also revealed an increased expression of S100A8 during intestinal inflammation in vivo. Following TWP intervention, the expression of S100A8 and TLR4 signaling pathways and inflammatory factors decreased. This was

accompanied by a reduction in disease activity index (DAI) score, alleviation of histopathology, and significant relief of colonic inflammatory activity.

Conclusion: S100A8 is the key gene in UC, which may regulate the TLR4 signaling pathway to mediate the UC inflammatory response. Additionally, S100A8 is located upstream of the TLR4 signaling pathway, and TWP has been shown to have a regulatory effect on this pathway.

Key words: ulcerative colitis, S100A8, TWP, TLR4 signaling pathway.

Conclusion

This study reveals the following findings: (1) S100A8 is a crucial gene involved in UC inflammation and serves as a significant target gene for TWP in regulating UC inflammation. (2) The use of TAK-242 to inhibit TLR4 activity revealed that S100A8 plays a role in regulating the TLR4 signaling pathway, specifically at the upstream stage of the inflammatory pathway. (3) TWP can down-regulate the expression of S100A8, inhibit the activation of TLR4 pathway, reduce the release of terminal inflammatory factors, and alleviate the intestinal inflammatory activities of UC rats.

Acknowledgments

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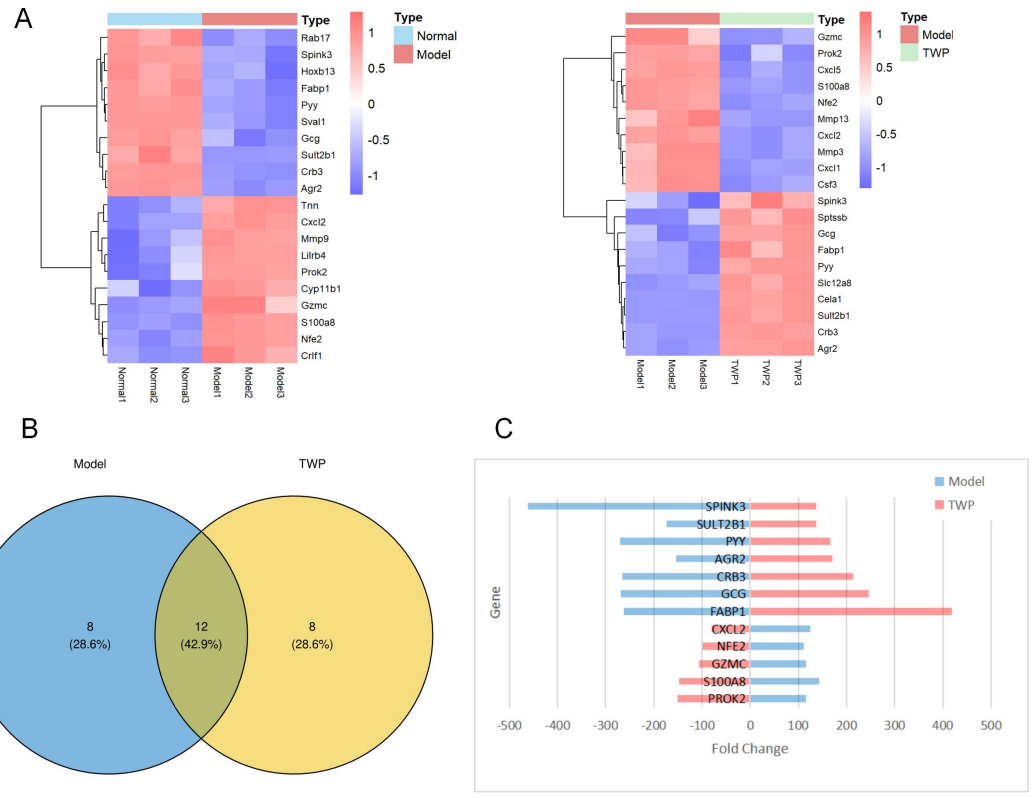

Fig 1. (A) The heat map of TOP 10 genes of normal control group vs model group and model group vs TWP high-dose group; (B) Venn diagram of the intersection of two groups of differential genes; (C) The fold difference of intersection genes in each

group.

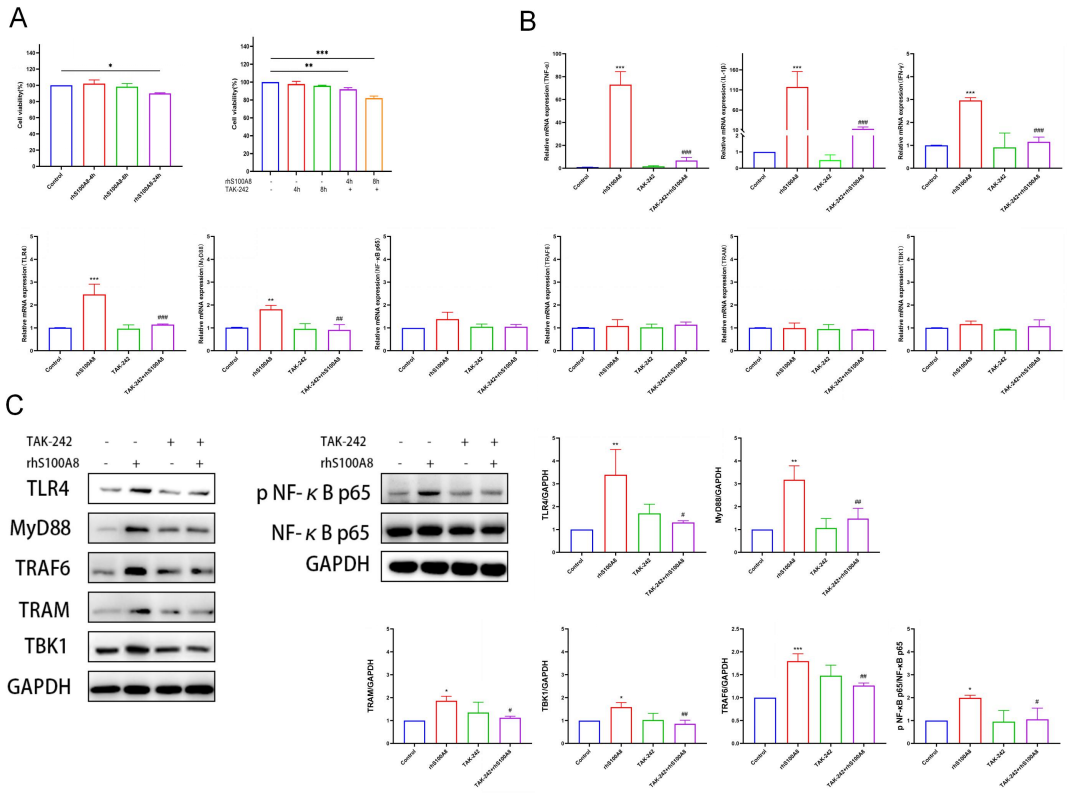

Fig 2. (A) CCK-8 detected cell viability; (B) qRT-PCR detected TLR4 Signaling pathway and terminal inflammatory factor mRNA expression; (C) WB detected the protein expression of each key node of TLR4 signaling pathway. The data are expressed as the mean ± SD of three independent experiments, compared with the Control group, *$P<0.05$, **$P<0.01$, ***$P<0.001$, compared with LPS group, #$P<0.05$, ##$P<0.01$, ###$P<0.001$.

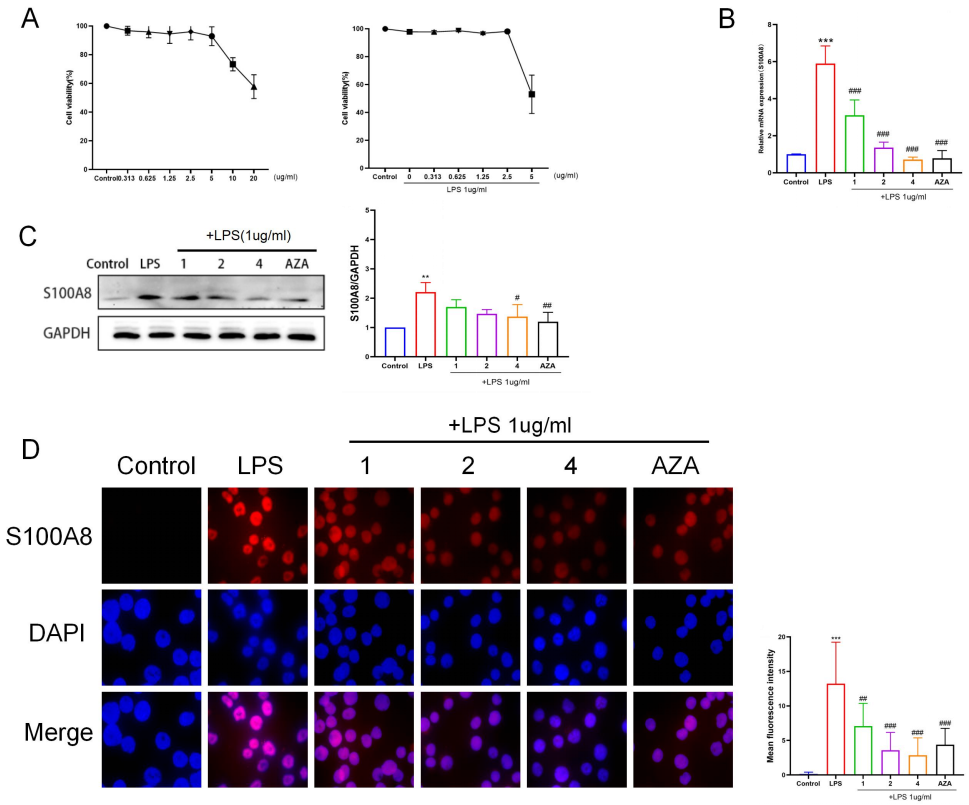

Fig 3. (A) CCK-8 detected cell viability; (B) S100A8 mRNA expression; (C) S100A8 protein expression; (D) S100A8 immunofluorescence intensity (magnification: 100X).

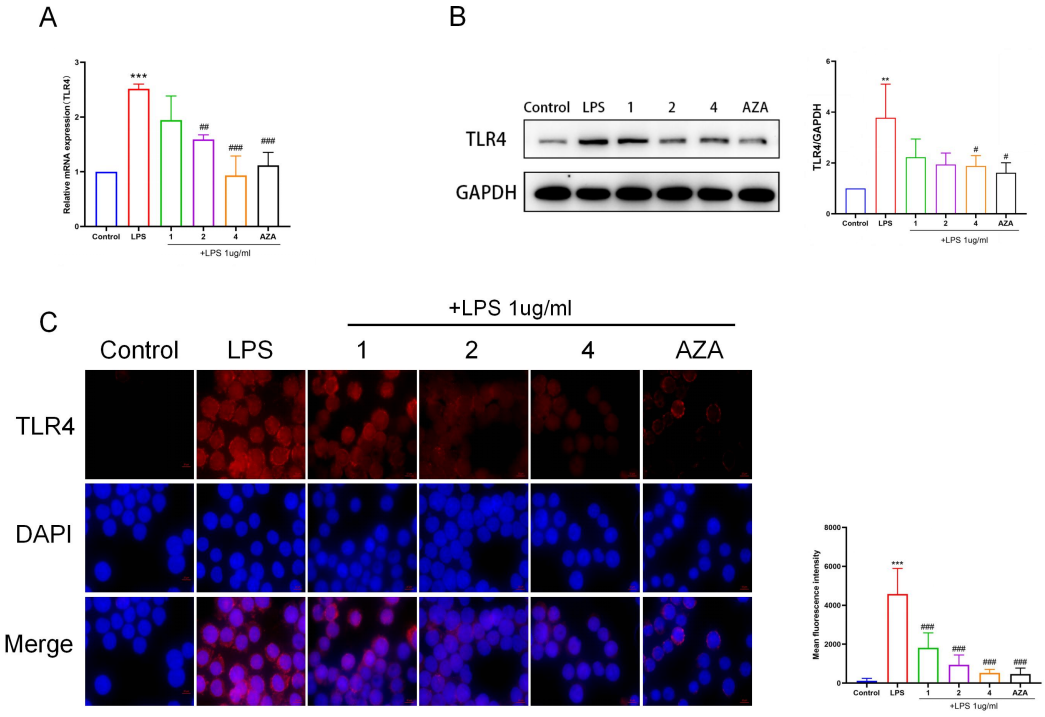

Fig 4. (A) qRT-PCR detection of TLR4 mRNA expression (B) WB detection of TLR4 protein; (C) TLR4 fluorescence intensity (magnification: 100X).

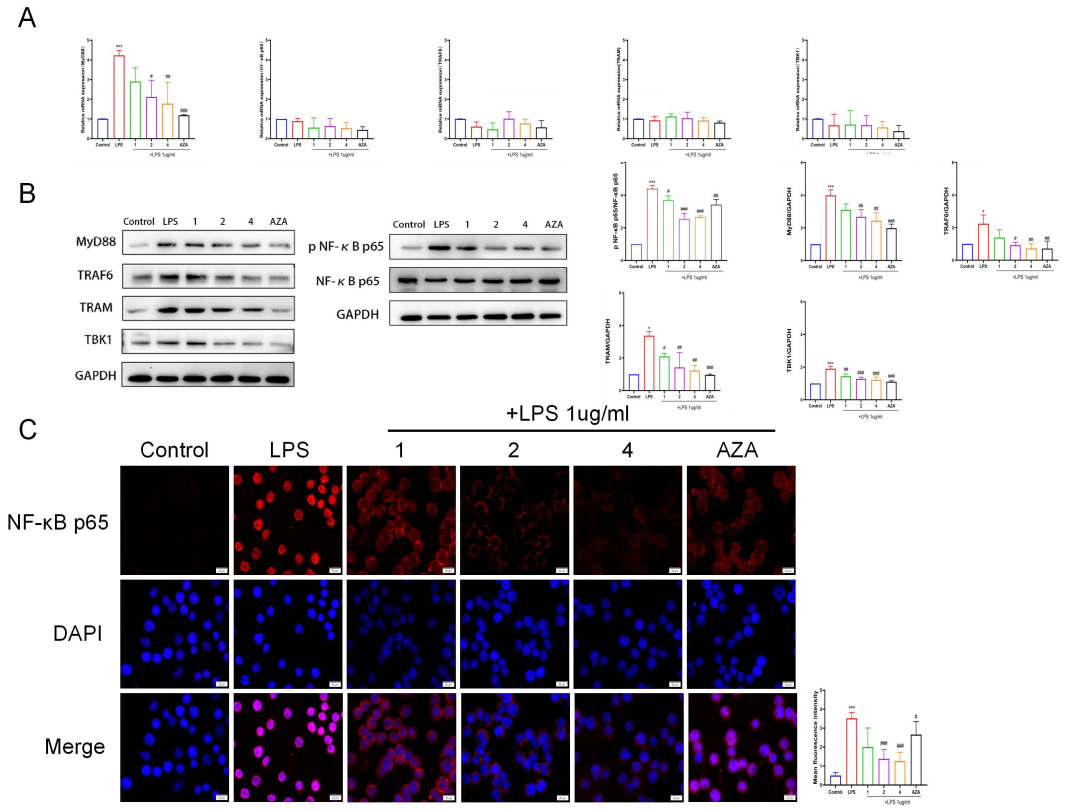

Fig 5. (A) mRNA expression changes of key nodes in TLR4 signaling pathway; (B) key node proteins in TLR4 signaling pathway; (C) NF- κ B p65 nuclear translocation changes.

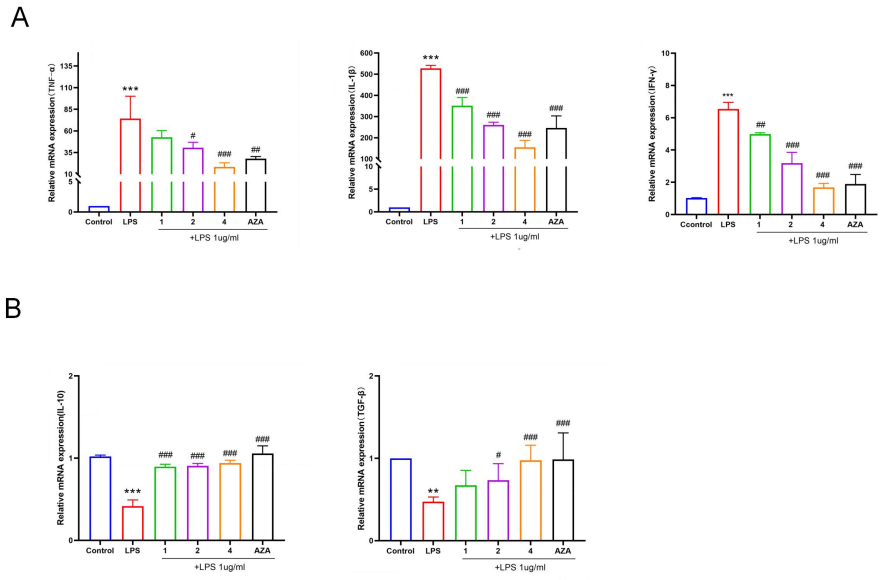

Fig 6. (A) TNF- α , IL-1 β , IFN- γ mRNA expression; (B) IL-10, TGF- β mRNA

expression.

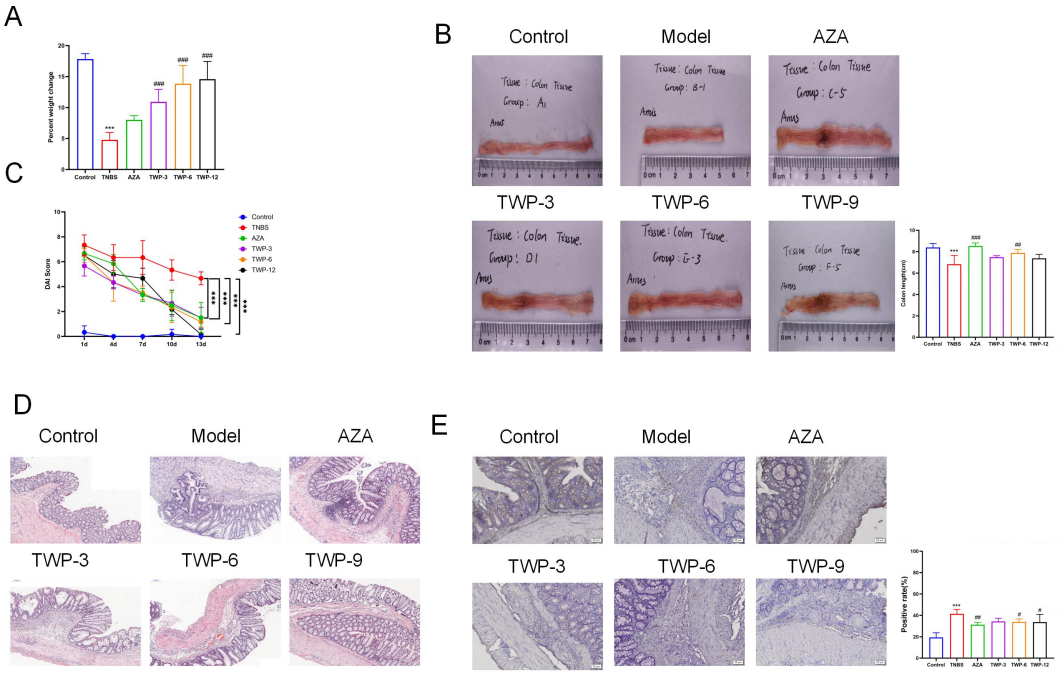

Fig 7. (A) Weight gain percentage of UC rats; (B) Colon length analysis; (C) DAI score change; (D) Colon HE staining (magnification: 10X); (E) Colon S100A8 immunohistochemical analysis (Magnification: 10X).