Genes & Diseases (2024) 11, 101092

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/en/journals/genes-diseases

RAPID COMMUNICATION

Epinephrine promotes tumor progression and M2 polarization of tumor-associated macrophages by regulating the TRIM2-NF-κB pathway in colorectal cancer cells

Tumor-associated inflammation is an important component of the tumor microenvironment, and an important factor affecting tumor progression. In the tumor microenvironment, tumor-associated macrophages (TAMs) receive different stimuli and can be polarized into classically activated M1 macrophages and alternatively activated M2 macrophages. Many studies have indicated that the polarization of TAMs is closely related to tumor progression. M2-polarized TAMs have been highly correlated with tumor metastasis, angiogenesis, and poor prognosis, whereas M1-polarized TAMs suppress tumor development. Tumors with higher densities of M2 macrophages and lower densities of M1 macrophages have poor clinical outcomes.[1] In terms of molecular mechanism, M2-polarized TAMs can produce various cytokines required for tumor cell growth and angiogenesis, including TGF-β, VEGF, and EGF.[2] In addition, M2-polarized TAMs can inhibit humoral and cellular immunity to cancer cells through various pathways, maintain tumor cells in an immune tolerance state, and evade clearance by the body. Tumor cells can regulate TAM polarization toward the M1 or M2 phenotype by regulating the tumor microenvironment and releasing cytokines.[3] TRIM family proteins have been reported to be closely related to immune regulation, inflammation, and tumorigenesis.[4] In our previously published research, we found that epinephrine (Epi) could promote the progression of colorectal cancer (CRC) by promoting the expression of TRIM2.[5] However, the immunomodulatory role of TRIM2 in CRC is still unknown. In this study, we found that Epi promotes tumor proliferation, migration, and M2 polarization of TAMs by up-regulating TRIM2 expression in CRC cells. TRIM2 expression is closely related to CRC progression. TRIM2 promotes the development of CRC by promoting the ubiquitination and degradation of IκBα. TRIM2 can also promote M2 polarization of TAMs, which can further promote the progression of CRC. Collectively, targeting the Epi/TRIM2 signaling pathway might be a promising treatment option for CRC.

Firstly, we found that TRIM2 expression was considerably increased in CRC tissues (Fig. 1A; Fig. S1A) and TRIM2 expression was strongly linked with tumor stage and lymph node metastasis (Fig. S1B, C). Next, we explored the probable relationship between TRIM2 and immune cell infiltration in the TIMER database. The results indicated that TRIM2 expression is associated with the infiltration of various immune cells including macrophages in CRC (Fig. S1D). The expression of iNOS and CD86 (M1 marker), CD206 and CD163 (M2 markers), and CD68 (macrophage markers) were investigated by immunohistochemistry in human CRC tissues. The results indicated that more M2 macrophage polarization was found in tumor tissues with high TRIM2 expression (Fig. 1B; Fig. S1E). Moreover, the expression of TRIM2 in several CRC cell lines was examined (Fig. S1F, G). Then, the function of TRIM2 in CRC was investigated, and the results indicated that the expression of TRIM2 related to the proliferation (Fig. 1C, D; Fig. S2G–Q) and migration (Fig. 1E; Fig. S2A–F, R) abilities of CRC cells *in vivo* and *in vitro*.

We further detected markers of TAM polarization in xenograft tumors by immunohistochemistry. We found TRIM2 may promote M2 polarization of TAMs (Fig. S3A–D). After activation with PMA, THP-1 cells were cocultured with treated CRC cells. Then, flow cytometric and immunofluorescence analyses were performed, and the results indicated that TRIM2 promoted M2 polarization of TAMs (Fig. 3F; Fig. S3E–H).

Liquid chromatography and high-throughput mass spectrometry analyses were applied to search proteins that

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2023.101092

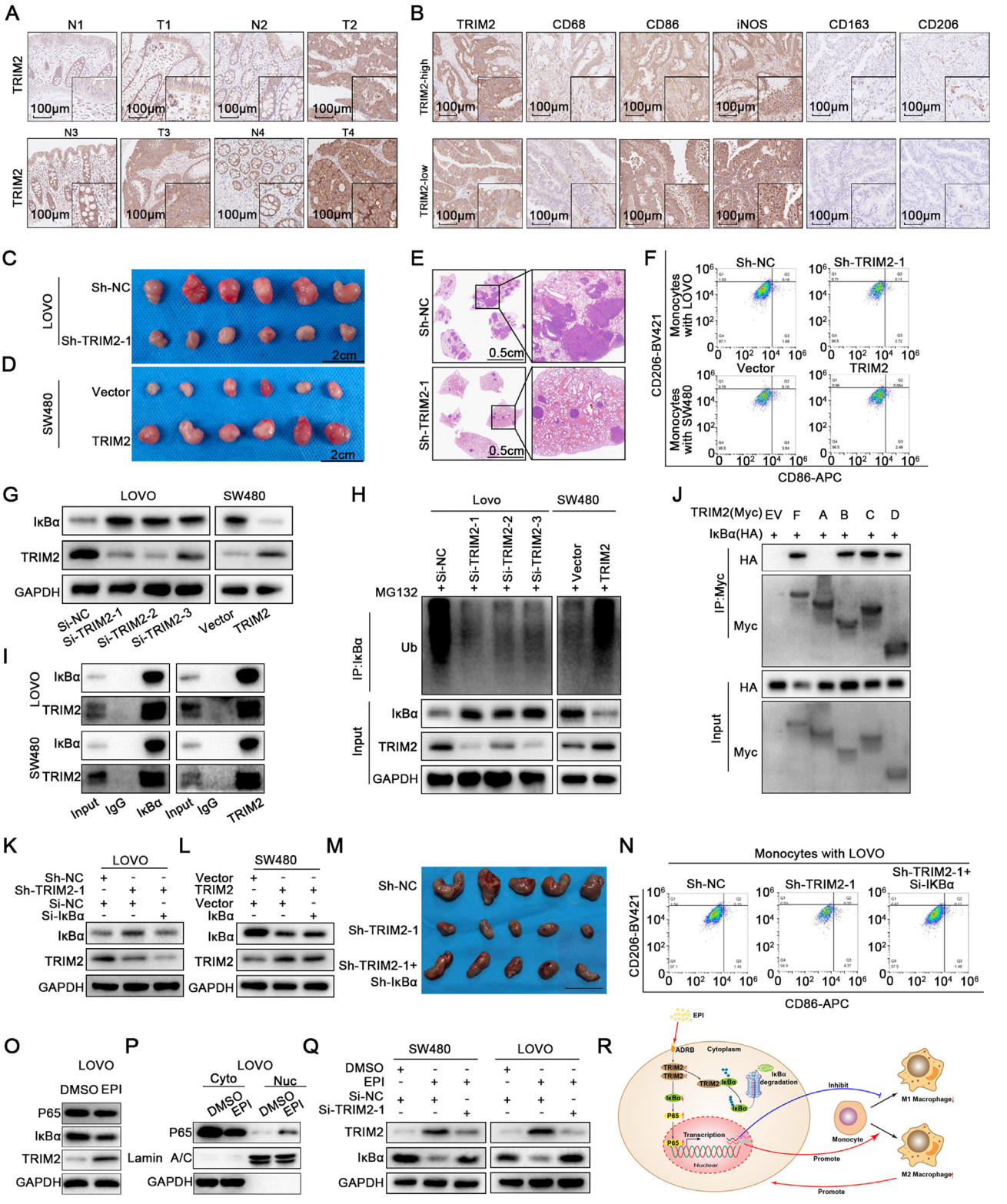

Figure 1 Epinephrine promotes tumor progression and M2 polarization of tumor-associated macrophages by regulating the TRIM2-NF-κB pathway in colorectal cancer (CRC) cells. **(A)** The expression of TRIM2 in CRC tissues was examined by immunohistochemistry ($n = 65$). **(B)** The protein expression of TRIM2, iNOS, and CD86 (M1 markers), CD206 and CD163 (M2 markers), and CD68 (macrophage marker) in human CRC tissues was evaluated by immunohistochemistry. **(C)** Subcutaneous xenograft tumors in the sh-TRIM2 group grew slower than those in the control group. **(D)** Subcutaneous xenograft tumors in the TRIM2 group grew faster than those in the control group. **(E)** Fewer lung metastatic nodules were observed in the sh-TRIM2 group than in the control group. **(F)** THP-1 cells were cocultured with treated CRC cells, and markers of TAM polarization in THP-1 cells were evaluated by flow cytometry ($n = 3$). **(G)** The expression of TRIM2 and IκBα in CRC cells transduced with sh-TRIM2 or transfected with the TRIM2

interact with TRIM2 (Table S2). Next, we predicted the top 300 substrates for TRIM2 through the Ubibrowser database (Table S3). The same genes between the two groups were explored (Fig. S4A). The results indicated that TRIM2 may bind to NFKBIA (IκBα), TRIM3, and ACTN4. Since previous studies have indicated TRIM acts as a ubiquitin ligase to regulate the NF-κB signaling pathway, IκBα was then selected to further investigation. We found that the expression of TRIM2 was negatively correlated with that of IκBα (Fig. S4B—D). Next, the results of Western blot analysis indicated that TRIM2 inhibited the expression of IκBα (Fig. 1G; Fig. S4E) and promoted the nuclear translocation of p65 (Fig. S4F—H) in CRC cells. After silencing or overexpressing TRIM2, CRC cells were further stimulated with cycloheximide. The results showed that TRIM2 may decrease IκBα protein stability (Fig. S4I—L). In addition, MG132 was used to treat cells. We found that TRIM2 induced IκBα degradation in a proteasome-dependent manner (Fig. S4M). Moreover, we further investigated the polyubiquitinated form of endogenous IκBα (Fig. 4H). We found that TRIM2 promoted the polyubiquitination of endogenous IκBα. Furthermore, plasmids expressing TRIM2, IκBα, Ub, Ubk48, or Ubk63 were transfected into 293T cells. We found that the total and K48-linked ubiquitination but not the K63-linked ubiquitination of IκBα was obviously increased by TRIM2, suggesting that TRIM2 promotes the K48-linked polyubiquitination of IκBα (Fig. S4N).

The results of Co-IP, GST pull-down assays, and immunofluorescence staining indicated that TRIM2 physically interacted with IκBα (Fig. 1I; Fig. S5A—D). We further constructed plasmids with the full-length TRIM2 sequence or sequences with the deletion of different domains of TRIM2. Co-IP was applied to explore the interaction of each truncation deletion with IκBα. We found that amino acids 1—111 participated in this interaction (Fig. 1J; Fig. S5E). Similarly, amino acids 181—317 of IκBα may participate in the interaction of IκBα with TRIM2 (Fig. S5F, G). Finally, the plasmids expressing full-length TRIM2 or TRIM2 with deletion of amino acids 1—111 were transfected into CRC cells, and we found that the amino acids 1—111 played an indispensable role in TRIM2-mediated inhibition of IκBα expression (Fig. S5H, I), promotion of P65 nuclear translocation (Fig. S5J, K), and promotion of IκBα degradation (Fig. S5L—P).

Rescue experiments were performed, and we found that silencing IκBα reversed the inhibitory effect of TRIM2 on IκBα expression (Fig. 1K, L; Fig. S6A, B), and silencing IκBα also significantly reversed the promoting effects of TRIM2 on P65 nuclear translocation (Fig. S6C—F), cell proliferation and metastasis (Fig. 1M; Fig. S6G—N), and M2 polarization of TAMs (Fig. 1N; Fig. S6O, P).

In previous studies, we proved that Epi can increase the level of TRIM2 in CRC. Therefore, we sought to determine whether Epi can also promote the M2 polarization of TAMs. We treated CRC cells with Epi (50 nM) *in vitro*. The results of Western blot analysis showed that Epi promoted the expression of TRIM2 and suppressed the expression of IκBα in CRC cells (Fig. 1O; Fig. S7A, B). Moreover, Epi promoted P65 nuclear translocation in CRC cells (Fig. 1P; Fig. S7C—E). Then, the function of Epi in CRC was investigated, and the results indicated that Epi can enhance the migration and proliferation abilities of CRC cells *in vivo* and *in vitro* (Fig. S7F—N). We also examined the levels of TRIM2 and macrophage markers in xenografts by immunohistochemistry, which revealed that Epi promoted TRIM2 expression and M2 macrophage polarization (Fig. S7O, P).

Finally, we down-regulated the expression of TRIM2 in cells treated with Epi, which suppressed the regulatory effects of Epi on IκBα expression (Fig. 1Q; Fig. S8A, B), migration (Fig. S8C—F), and proliferation (Fig. S8G, H), indicating that Epi promotes the proliferation and metastasis of CRC cells and M2 polarization of THP1 cells by up-regulating the expression of TRIM2 in CRC cells (Fig. 1R).

In this study, we revealed the role of TRIM2 in regulating CRC progression and promoting TAM polarization. TRIM2 expressed in CRC cells promotes TAM polarization toward the M2 phenotype. TRIM2 directly binds to IκBα and induces the degradation of IκBα by ubiquitination, thereby activating the NF-κB signaling pathway. In summary, we revealed a unique role for TRIM2 in controlling TAM polarization and tumor growth in CRC. These data imply that targeting the TRIM2 signaling pathway might be a promising therapeutic approach for CRC.

Ethics declaration

Our study for animal (S2501) and human (S-123/2022) experiments was approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China).

Author contributions

Zhengyi Liu: conceptualization, formal analysis, investigation, funding acquisition, writing — review, and editing.

overexpression plasmid was measured by Western blot. **(H)** Co-IP showing the effect of TRIM2 expression changes on IκBα polyubiquitination in LoVo and SW480 cells. **(I)** Co-IP showing the interaction of endogenous TRIM2 and IκBα in LoVo and SW480 cells. **(J)** Immunoprecipitation of TRIM2 constructs and IκBα in 293T cells. **(K)** The expression of IκBα in LoVo cells transfected with sh-TRIM2 was inhibited by si-IκBα, and TRIM2 and IκBα expression were measured by Western blot. **(L)** The expression of IκBα in SW480 cells transfected with the TRIM2 overexpression plasmid was up-regulated by the IκBα plasmid, and TRIM2 and IκBα expression were measured by Western blot. **(M)** Silencing IκBα significantly reversed the promoting effect of TRIM2 on cell proliferation *in vivo* (scale bar = 2 cm). **(N)** Silencing IκBα significantly reversed the promoting effect of TRIM2 on the M2 polarization of TAMs ($n = 3$). **(O)** Western blot analysis revealed that Epi promoted the expression of TRIM2 and inhibited the expression of IκBα in LoVo cells. **(P)** Western blot analysis of cytoplasmic (Cyto) and nuclear (Nuc) fractions isolated from the indicated cells. Epi promoted P65 nuclear translocation in LoVo cells. **(Q)** Western blot analysis indicated that inhibiting TRIM2 expression reversed the Epi-induced promotion of IκBα expression in CRC cells. **(R)** The model of epinephrine promotes tumor progression and M2 polarization of tumor-associated macrophages by regulating the TRIM2-NF-κB pathway in CRC cells. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Chenxing Jian: conceptualization, formal analysis, investigation, and methodology. Wenzheng Yuan: formal analysis, investigation, and funding acquisition. Guiqing Jia: investigation, methodology, editing, and funding acquisition. Donghui Cheng: formal analysis, methodology, and funding acquisition. Yanzhuo Liu, Yanling Zhang, and Bin Zhang: formal analysis, investigation, and methodology. Zili Zhou and Gaoping Zhao: conceptualization, funding acquisition, supervision, writing — review, and editing.

Conflict of interests

The authors declare that they have no conflict of interests.

Funding

This study was supported by the National Natural Science Foundation of China (No. 81902435), Natural Science Foundation of Fujian Province of China (No. 2021J011371), Sichuan Science and Technology Program of China (No. 2021YFS0375, 2023NSFSC1899), Science and technology project of Henan Province, China (No. 222102310037), Henan Province Medical Science and Technology Project of China (No. LHGJ20220018), Medjaden Academy & Research Foundation for Young Scientists (China) (No. MJR20221014), and Project of Sichuan Provincial People's Hospital (China) (No. 2022QN03, 2021LY14).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.101092.

References

1. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. *Cell*. 2010;141(1):39—51.
2. Denome RM, Cole CN. Patterns of polyadenylation site selection in gene constructs containing multiple polyadenylation signals. *Mol Cell Biol*. 1988;8(11):4829—4839.
3. Chanmee T, Ontong P, Konno K, Itano N. Tumor-associated macrophages as major players in the tumor microenvironment. *Cancers*. 2014;6(3):1670—1690.
4. Kawai T, Akira S. Regulation of innate immune signalling pathways by the tripartite motif (TRIM) family proteins. *EMBO Mol Med*. 2011;3(9):513—527.
5. Zhou Z, Shu Y, Bao H, et al. Stress-induced epinephrine promotes epithelial-to-mesenchymal transition and stemness of CRC through the CEBPB/TRIM2/P53 axis. *J Transl Med*. 2022;20(1):262.

Zhengyi Liu [a,b,1], Chenxing Jian [c,1], Wenzheng Yuan [d,1], Guiqing jia [e], Donghui cheng [f], Yanzhuo Liu [e], Yanling Zhang [e], Bin Zhang [e], Zili Zhou [c,e,*], Gaoping Zhao [e,*]

[a] *Department of Breast Surgery, Henan Provincial People's Hospital, The People's Hospital of Zhengzhou University, The People's Hospital of Henan University, Zhengzhou, Henan 450003, China*

[b] *Microbiome Laboratory, Henan Provincia People's Hospital Zhengzhou University People's Hospital, Zhengzhou, Henan 450000, China*

[c] *Department of Gastrointestinal Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430022, China*

[d] *Department of Gastrointestinal Surgery II, Renmin Hospital of Wuhan University, Wuhan, Hubei 430060, China*

[e] *Department of Gastrointestinal Surgery, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, Sichuan 610072, China*

[f] *Department of Hepatobiliary Surgery, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, Sichuan 610072, China*

*Corresponding authors. Fax: +28 87393778.
E-mail addresses: zhouzili@med.uestc.edu.cn (Z. Zhou), gzhao@uestc.edu.cn (G. Zhao)

5 November 2022
Available online 14 September 2023

[1] These authors contributed equally to this work.