Research Article

Tanshinone IIA May Inhibit Gastric Cancer via Affecting the Intestinal Microbiome

Fenggang Lu, Yi Zhang, Pinghui Song, Yu Xu, Zhongkun Wu, Qinyu Wang, and Naiying Shen

Department of General Surgery, Shaanxi Health Care Group 215 Hospital, Shaanxi 712000, China

Correspondence should be addressed to Naiying Shen; shennaiying_edu@outlook.com

Received 24 August 2022; Revised 5 September 2022; Accepted 9 September 2022; Published 26 September 2022

Academic Editor: Liaqat Ali

Copyright © 2022 Fenggang Lu et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Gastric cancer (GC) belongs to a type of the most deadly cancer in the world, and the incidence rate of GC will increase in the coming decades [1, 2]. In spite of the recent advances in therapy, the long-time overall survival rate of GC is inferior to 10% [3, 4]. As a consequence, studying more methods for GC diagnosis and therapy is necessary.

Previous studies have proposed the possible role of gut microbiome in cancers [5, 6]. Intestinal dysbacteriosis, which is abnormal changes in the gut microbiota, may induce abnormal immune reaction in the gastric tissues, triggering the chronic inflammation and epithelial-mesenchymal transition (EMT) of the epithelial cells, ultimately stimulating tumor development [7]. Therefore, to alleviate the intestinal dysbacteriosis condition may be another way to treat various types of cancers.

Tanshinone IIA (Tan IIA) was an active component that separated from Danshen (a traditional medication) [8, 9]. Tan IIA has antiapoptotic, antioxidant, and anticoagulant along with other effects and has been extensively utilized in treating cardiovascular as well as cerebrovascular diseases. Recently, the antitumor potentials of Tan IIA have been discussed in many previous studies, including cancers [10]. Interestingly, a previous study showed that the major bioactive parts of Danshen are tanshinones, which can exert medical effect in the rat chronic renal failure model by modulating the intestinal microbiome along with alleviating the intestinal dysbacteriosis [11]; moreover, tanshinol borneol ester, which is a bioactive component isolated from Danshen, can reverse the intestinal dysbacteriosis condition of high-fat diet-induced mouse obesity model [12]; furthermore, Danshen has been reported to restore the balance of intestinal microbiome and reduce the translocation of the

1. Background

Gastric cancer (GC) belongs to a type of the most deadly cancer in the world, and the incidence rate of GC will increase in the coming decades [1, 2]. In spite of the recent advances in therapy, the long-time overall survival rate of GC is inferior to 10% [3, 4]. As a consequence, studying more methods for GC diagnosis and therapy is necessary.

Previous studies have proposed the possible role of gut microbiome in cancers [5, 6]. Intestinal dysbacteriosis, which is abnormal changes in the gut microbiota, may induce abnormal immune reaction in the gastric tissues, triggering the chronic inflammation and epithelial-mesenchymal transition (EMT) of the epithelial cells, ultimately stimulating tumor development [7]. Therefore, to alleviate the intestinal dysbacteriosis condition may be another way to treat various types of cancers.

Tanshinone IIA (Tan IIA) was an active component that separated from Danshen (a traditional medication) [8, 9]. Tan IIA has antiapoptotic, antioxidant, and anticoagulant along with other effects and has been extensively utilized in treating cardiovascular as well as cerebrovascular diseases. Recently, the antitumor potentials of Tan IIA have been discussed in many previous studies, including cancers [10]. Interestingly, a previous study showed that the major bioactive parts of Danshen are tanshinones, which can exert medical effect in the rat chronic renal failure model by modulating the intestinal microbiome along with alleviating the intestinal dysbacteriosis [11]; moreover, tanshinol borneol ester, which is a bioactive component isolated from Danshen, can reverse the intestinal dysbacteriosis condition of high-fat diet-induced mouse obesity model [12]; furthermore, Danshen has been reported to restore the balance of intestinal microbiome and reduce the translocation of the
The transcriptional factor nuclear factor-κB (NF-κB) family regulates a huge number of genes implicated in different cellular processes, such as cell proliferation, differentiation, and innate and adaptive immune responses. The NF-κB family consists of 5 members, p50, p52, p65, RelB, and c-Rel, which interact to form homopolymers or heterodimers [14]. Activation of the NF-κB signaling pathway contributes to the progression of several cancers, GC included [15]. Moreover, the NF-κB signaling pathway plays a key role in the host response to microbial infection by coordinating innate and adaptive immune functions [16]. Since Tan IIA is an important bioactive component of the Danshen, according to the mentioned previous reports, we speculated whether Tan IIA may also exert its therapeutic effects in a disease with intestinal dysbacteriosis, at least partially, via regulating the intestinal microbiome. Nevertheless, it is obscure whether Tanshinone IIA affects the intestinal dysbacteriosis and plays antitumor roles. Herein, we explored Tanshinone IIA role on the intestinal dysbacteriosis of GC xenograft mice and the underlying mechanism. Our work may offer a new mechanism along with an alternative treatment strategy for GC patients.

2. Methods

2.1. Cell Cultivation. Procell (Wuhan, China) supplied human gastric cell line HT29 (Cat. No. TCHu103), which were hatched in McCoy’s 5A medium with 10% FBS at 37°C with 5% CO₂.

2.2. Xenograft Tumor Models of HT29 Cells and Intestinal Dysbacteriosis Mouse Model. 40 male mice (4-6 weeks old) from Animal Center of Nanjing Medical University (Nanjing, China) were spanulated into 4 groups (n = 10 every group) in random (control group, Tan IIA high group, Tan IIA +dysbacteriosis group, and dysbacteriosis group). All procedures were conducted following the Guidelines for the Care and Use of Laboratory Animals with the approval of the Ethics Committee of the Shaanxi Health Care Group 215 Hospital. The mice were placed in a specific pathogen-free (SPF) condition, supplying water and food. For the xenograft tumor models, HT29 cells (2 x 10⁶ cells) with different treatments were subcutaneously injected into the mice. For Tan IIA treatment, low and high doses of Tan IIA were 10 mg/kg and 30 mg/kg, respectively. 21 days later, the mice were treated with isoflurane for anesthetizing and placed in a room with CO₂ (flow rate < 30% volume/min) for 7 minutes for sacrifice. After excising the tumor tissues, the tumor was measured.

An intestinal dysbiosis mouse model has been built as previously documented [17]. Mice have been orally injected by ampicillin (1 g/l), vancomycin (0.5 g/l), neomycin (1 g/l), and metronidazole (1 g/l) to create intestinal dysbiosis condition.

2.3. Western Blotting. Total proteins were extracted and then isolated by a 10% SDS–PAGE. After shifting to PVDF membranes along with sealing in TBST solution including 5% skimmed milk, the membranes were hatched with primary antibodies including p-NF-κB (Abcam, ab239882, 1/1000) and β-actin (Abcam, ab8226, 1 µg/ml) and then hatched with HRP-conjugated secondary antibodies (Abcam, ab239882, 1/2000). Protein bands were examined via enhanced chemiluminescence detection system (Millipore, USA). Images are revealed as representatives of three independent assays.

2.4. ELISA Assay. The levels of interleukin-6 and interleukin-1β were measured by ELISA methods (Beyotime, Shanghai, China).

2.5. Statistical Analysis. All assays were implemented in triplicate. Data were indicated as mean ± SD and programed via SPSS 19.0 software. Comparison was assessed using unpaired Student’s t-test. p value below 0.05 was meaningful.

3. Results

3.1. Changes of the Microbiome in the Intestinal of the Tumor Mice in Xenograft Tumor. First, we established the xenograft tumor mouse models, and the microbiome in the intestinal of the tumor mice and normal mice were compared. As illustrated in Table 1, the relative abundance of Lactobacillus as well as Bacteroides decreased (p < 0.01) and Escherichia as well as Candidatus markedly increased (p < 0.001) in tumor mice.

3.2. Tan IIA Hinders the Growth of Xenograft Tumor and Change the Microbiome in the Intestinal. Furthermore, to verify whether Tan IIA could repress the growth of xenograft tumor via modulating the microbiome in the intestinal, we treated Tan IIA into the xenograft tumor mice. As unveiled in Figure 1, the tumor size and weight were markedly decreased in the Tan IIA low and Tan IIA high groups relative to the control group (p < 0.05 and p < 0.01). At the same time, the tumor volume was gradually reduced at 14 and 21 days (p < 0.05 and p < 0.01). Moreover, Tan IIA elevated the abundance of Lactobacillus as well as Bacteroides and declined the abundance of Escherichia as well as Candidatus in a dose-dependent way (Table 2, p < 0.05 and p < 0.01).

3.3. Intestinal Dysbacteriosis Condition Partially Blocked Tan IIA-Stimulated Antitumor Effects. Next, an intestinal dysbiosis model was established by a large dose of antibiotics as

| Table 1: Comparison of the abidance of intestinal microbiome between the GC tumor mice and the control mice. |
|---------------------------------|-----------------|-----------------|
| | Control | Tumor | p value |
| Lactobacillus | 33.24 ± 2.86 | 13.51 ± 1.35 | p < 0.01 |
| Bacteroides | 32.05 ± 2.33 | 19.25 ± 1.86 | p < 0.01 |
| Escherichia | 0.01 ± 0.01 | 2.21 ± 0.13 | p < 0.001 |
| Candidatus | 2.09 ± 0.24 | 8.25 ± 0.32 | p < 0.001 |
previously described, and as Figure 2 showed, Tan IIA decreased the tumor growth; meanwhile, intestinal dysbiosis condition could partially block Tan IIA caused inhibition on the tumor growth in xenograft tumor mice ($p < 0.05$ and $p < 0.01$).

### 3.4. Intestinal Dysbacteriosis Abrogated Tan IIA-Stimulated Decrease in the NF-κB Signaling in Xenograft Tumor Mice.

Finally, to elucidate the possible mechanism of Tan IIA-induced antitumor effects via regulating the microbiome in the intestinal, the phosphorylation of the NF-κB in rats of different treatment and expressions of the downstream cytokines IL-6 and IL-1β were assessed by WB methods. We found Tan IIA lead to decline in the expression of p-p65-NF-κB, IL-6, and IL-1β of the tumor samples in comparison with the controls (Figure 3, $p < 0.01$); meanwhile, intestinal dysbacteriosis condition can increase the phosphorylation of p65, IL-6, and IL-1β in Tan IIA-treated mice (Figure 3, $p < 0.01$).

### 4. Discussion

GC is deemed to be a leading cause of death worldwide [18, 19]. Although patients with GC have benefited from chemotherapy with the improved overall survival, current chemotherapeutic agents have serious side effects in patients. Therefore, the discovery and development of the specific molecular targets necessitate modulation in anticancer therapy.

The role of the gut microbiome in a variety of cancers have been documented in many former studies, and now, intestinal dysbacteriosis has been identified as an important
reason in the development of cancers [20, 21]. In the present work, we discovered that the intestinal microbiome significantly altered in the GC tumor mice in comparison with the control mice, while the beneficial microorganism *Lactobacillus* as well as *Bacteroides* decreased, and the harmful microorganism *Escherichia* as well as *Candidatus* increased. These results were consistent with previous observation [22], suggesting that intestinal dysbacteriosis may contribute to the pathogenesis of GC.

The antitumor influences of Tan IIA have been discussed in many previous studies. For example, Tan IIA inhibits ovarian cancer growth by suppressing malignant properties [23]. Tan IIA reduces colorectal cancer cell viability by activating JNK-Mf signaling pathways [24]. More importantly, Tan IIA has been also reported to hinder GC progression [25] but reports on the potential of Tan IIA in the gut microbiome of GC is rare. In this work, we first elucidated that the potential Tan IIA in the intestinal microbiome. It was discovered that Tan IIA elevated the abundant of the useful microorganism whereas lessened the abundance of baleful microorganism in GC mice, and interestingly, when intestinal dysbacteriosis condition was created in Tan IIA-treated mice, the antitumor effects of Tan IIA has been partially blocked. Taken together, the above data mirrored that Tan IIA could exert its anti-GC influences in part via modulating the intestinal microbiome.

Intestinal dysbacteriosis was famous to trigger chronic inflammatory condition and consequentially contribute to the tumorigenesis. NF-κB was known as a complex of proteins that take part in the progression of immune responses [26, 27]. As reported before, the NF-κB pathway is involved in the regulation of intestinal microbiome in many diseases, such as colitis [28] and colorectal cancer [29], and its downstream cytokine IL-6 and IL-1β are closely related to the gut microbiota of GC [30]. Consistent with the above studies, the current work found that Tan IIA weakened p65-NF-κB phosphorylation along with the downstream cytokine IL-6 and IL-1β in GC tumors, and intestinal dysbacteriosis could partially block the anti-inflammatory influences of Tan IIA. These outcomes suggested that Tan IIA may influence the intestinal microbiome via modulation of the NF-κB signaling.

There are several limitations in our study. First, the potential mechanism of Tan IIA on regulating the NF-κB pathway was not further explored. In addition, the type of gut microbiota examined in our study was few. Therefore,
more researches are needed to further explore the clinical value of Tan IIA in GC.

5. Conclusion

In a word, we firstly reported that Tan IIA could hinder GC tumor growth via modulating the intestinal microbiome, maybe through inactivating the NF-κB signaling. Our work may provide basis for the using of Tan IIA as a useful medication for GC therapy.

Abbreviations

GC: Gastric cancer  
Tan IIA: Tanshinone IIA  
EMT: Epithelial-mesenchymal transition  
FBS: Fetal bovine serum  
SPF: Specific pathogen-free  
PBS: Phosphate buffer saline  
RIPA: Radio immunoprecipitation assay  
ELISA: Enzyme-linked immunosorbent assay  
IL-6: Interleukin-6  
IL-1β: Interleukin-1β  
NF-κB: Nuclear factor kappa-B.

Data Availability

Data included in the present study are available from the corresponding author under reasonable requests.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] W. Sun, X. Wang, D. Wang et al., "CD40×HER2 bispecific antibody overcomes the CCL2-induced trastuzumab resistance in HER2-positive gastric cancer," Journal for Immunotherapy of Cancer, vol. 10, no. 7, article e005063, 2022.

[2] A. Quinzii, C. Zecchetto, S. Casalino et al., "Clinical response to futibatinib in patients with high-level FGFR2-amplified advanced gastric cancer: two case reports," Clinical Drug Investigation, vol. 42, no. 8, pp. 697–701, 2022.

[3] Z. Zhang, B. Weng, Y. Qiu et al., "Effect of perioperative interleukin-6 and tumor necrosis factor-α on long-term outcomes in locally advanced gastric cancer: results from the CLASS-01 trial," Journal of Immunology Research, vol. 2022, Article ID 7863480, 11 pages, 2022.
[4] S. Yamada, O. Dohi, A. Harusato et al., “Endoscopic submucosal dissection for early gastric cancer in patients aged 85 years old or older is associated with a good prognosis compared to conservative treatment without any invasive procedure,” Digestion, vol. 103, no. 5, pp. 386–396, 2022.

[5] M. A. W. Khan, G. Ologun, R. Arora, J. L. McQuade, and J. A. Wargo, “Gut microbiome modulates response to cancer immunotherapy,” Digestive Diseases and Sciences, vol. 65, no. 3, pp. 885–896, 2020.

[6] W. Li, Y. Deng, Q. Chu, and P. Zhang, “Gut microbiome and cancer immunotherapy,” Cancer Letters, vol. 447, pp. 41–47, 2019.

[7] S. Xu, Z. Liu, M. Lv, Y. Chen, and Y. Liu, “Intestinal dysbiosis promotes epithelial-mesenchymal transition by activating tumor-associated macrophages in ovarian cancer,” Pathogens and Disease, vol. 77, no. 2, 2019.

[8] Z. Liu, W. Zhu, X. Kong et al., “Tanshinone IIA inhibits glucose metabolism leading to apoptosis in cervical cancer,” Oncology Reports, vol. 42, no. 5, pp. 1893–1903, 2019.

[9] L. Yang, G. Zhou, J. Liu et al., “Tanshinone I and Tanshinone IIA/B attenuate LPS-induced mastitis via regulating the NF-κB,” Biomedicine & Pharmacotherapy, vol. 137, article 111353, 2021.

[10] J. Qian, Y. Cao, J. Zhang et al., “Tanshinone IIA induces autophagy in colon cancer cells through MEK/ERK/mTOR pathway,” Translational Cancer Research, vol. 9, no. 11, pp. 6919–6928, 2020.

[11] L. L. Hong, Y. Zhao, C. Y. Yang et al., “Identification of chemical constituents in vitro and in vivo of Er Shen Zhenwu decoction by utilizing ultra-high-performance liquid chromatography with quadrupole time-of-flight mass spectrometry,” Journal of Separation Science, vol. 44, no. 24, pp. 4327–4342, 2021.

[12] P. Xu, F. Hong, J. Wang et al., “DBZ is a putative PPARγ agonist that prevents high fat diet-induced obesity, insulin resistance and gut dysbiosis,” Biochimica et Biophysica Acta - General Subjects, vol. 1861, no. 11, pp. 2690–2701, 2017.

[13] H. C. Xing, L. J. Li, K. J. Xu et al., “Effects of Salvia miltiorrhiza on intestinal microflora in rats with ischemia/reperfusion liver injury,” Hepatobiliary & Pancreatic Diseases International, vol. 4, no. 2, pp. 274–280, 2005.

[14] Y. Fan, R. Mao, and J. Yang, “NF-κB and STAT3 signaling pathways collaboratively link inflammation to cancer,” Protein & Cell, vol. 4, no. 3, pp. 176–185, 2013.

[15] J. X. Zhang, Z. H. Chen, D. L. Chen et al., “LINC01410-miR-532-NCF2-NF-κB feedback loop promotes gastric cancer angiogenesis and metastasis,” Oncogene, vol. 37, no. 20, pp. 2660–2675, 2018.

[16] C. Peng, Y. Ouyang, N. Lu, and N. Li, “The NF-κB signaling pathway, the microbiota, and gastrointestinal tumorigenesis: recent advances,” Frontiers in Immunology, vol. 11, p. 1387, 2020.

[17] G. Wan, M. Xie, H. Yu, and H. Chen, “Intestinal dysbiosis activates tumor-associated macrophages to promote epithelial-mesenchymal transition of colorectal cancer,” Innate Immunity, vol. 24, no. 8, pp. 480–489, 2018.

[18] H. Song, J. Y. Park, J. H. Kim et al., “Establishment of patient-derived gastric cancer organoid model from tissue obtained by endoscopic biopsies,” Journal of Korean Medical Science, vol. 37, no. 28, article e220, 2022.

[19] J. Zhang, M. Gao, Y. Niu, and J. Sun, “Identification of a novel ferropotis inducer for gastric cancer treatment using drug repurposing strategy,” Frontiers in Molecular Biosciences, vol. 9, article 860525, 2022.

[20] J. Guiot, I. Struman, E. Louis, R. Louis, M. Malaise, and M. S. Njock, “Exosomal miRNAs in lung diseases: from biologic function to therapeutic targets,” Journal of Clinical Medicine, vol. 8, no. 9, p. 1345, 2019.

[21] D. N. Jackson and A. L. Theiss, “Gut bacteria signaling to mitochondria inintestinal inflammation and cancer,” Gut Microbes, vol. 11, no. 3, pp. 285–304, 2020.

[22] P. P. Erawijantari, S. Mizutani, H. Shiroma et al., “Influence of gastrectomy for gastric cancer treatment on faecal microbiome and metabolome profiles,” Gut, vol. 69, no. 8, pp. 1404–1415, 2020.

[23] J. Zhou, Y. Y. Jiang, X. X. Wang et al., “Tanshinone IIA suppresses ovarian cancer growth through inhibiting malignant properties and angiogenesis,” Annals of Translational Medicine, vol. 8, no. 20, p. 1295, 2020.

[24] S. Jieensinu, H. Zhu, G. Li, K. Dong, M. Liang, and Y. Li, “Tanshinone IIA reduces SW837 colorectal cancer cell viability via the promotion of mitochondrial fission by activating JNK-Mff signaling pathways,” BMC Cell Biology, vol. 19, no. 1, p. 21, 2018.

[25] Z. Guan, J. Chen, X. Li, and N. Dong, “Tanshinone IIA induces ferropotis in gastric cancer cells through p53-mediated SLC7A11 down-regulation,” Bioscience Reports, vol. 40, no. 8, 2020.

[26] Z. Liu, Y. Huang, Y. Jiao et al., “Polystyrene nanoplastic induces ROS production and affects the MAPK-HIF-1/NFκB-mediated antioxidant system in Daphnia pulex,” Aquatic Toxicology, vol. 220, article 105420, 2020.

[27] A. Oekkingshaus, M. S. Hayden, and S. Ghosh, “Crosstalk in NF-κB signaling pathways,” Nature Immunology, vol. 12, no. 8, pp. 695–708, 2011.

[28] Y. Qu, X. Li, F. Xu et al., “Kaempferol alleviates murine experimental colitis by restoring gut microbiota and inhibiting the LPS-TLR4-NF-κB axis,” Frontiers in Immunology, vol. 12, article 679897, 2021.

[29] C. Guo, D. Guo, L. Fang et al., “Ganoderma lucidum polysaccharide modulates gut microbiota and immune cell function to inhibit inflammation and tumorigenesis in colon,” Carbohydrate Polymers, vol. 267, article 118231, 2021.

[30] F. Chu, Y. Li, X. Meng et al., “Gut microbial dysbiosis and changes in fecal metabolic phenotype in precancerous lesions of gastric cancer induced with N-methyl-N-nitro-N-nitrosoguanidine, sodium salicylate, ranitidine, and irregular diet,” Frontiers in Physiology, vol. 12, article 733979, 2021.