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Experimental Study on the Effect of Modified Tianma Granules on Colorectal Cancer Cell Line HCT116

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Abstract: *Objective:* To investigate the mechanism of modified Tianma Granules (mTMG) in inducing apoptosis of colorectal cancer HCT116 cells through regulating the PTEN/PI3K/AKT/mTOR signaling pathway and enhancing NKG2D expression based on the "toxicity-deficiency" theory of traditional Chinese medicine. *Methods:* Drug-containing sera were prepared from SD rats administered with mTMG formulations: the blank group (normal saline), detoxification group (detoxification components), tonifying deficiency group (tonic components), and original prescription group (all components). HCT116 cells were treated with 20% drug-containing serum for 48 h. Apoptosis was detected by Annexin V-FITC/PI double staining flow cytometry. CD56 and CD107a expression in NK92 cells were measured by flow cytometry. PTEN and NKG2D protein levels were determined by Western blot. *Results:* The apoptosis rate in the original prescription group (11.60% ± 1.40%) was significantly higher than that in the blank control group (1.70% ± 0.78%) and blank serum group (2.01% ± 0.04%) ($P < 0.05$). PTEN expression (0.86 ± 0.11) and NKG2D expression (0.64 ± 0.08) in the original prescription group were significantly higher than those in the blank control group (0.42 ± 0.05 and 0.39 ± 0.04) ($P < 0.05$). CD56 (5.22% ± 0.34%) and CD107a (15.56% ± 0.49%) expression in the detoxification group were significantly higher than those in the blank control group (1.32% ± 0.33% and 5.48% ± 0.42%) ($P < 0.05$). *Conclusion:* mTMG can induce HCT116 cell apoptosis by up-regulating PTEN to inhibit the PI3K/AKT/mTOR pathway and enhancing NKG2D to activate NK cell cytotoxicity, with synergistic effects between detoxification and tonifying deficiency components.

Keywords: Tianma Granules; HCT116; PTEN; NKG2D; apoptosis

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1. Introduction

Colorectal cancer (CRC) is the third most common malignancy worldwide and the second leading cause of cancer-related deaths, with approximately 900,000 deaths annually [1,2]. Surgery, radiotherapy, and chemotherapy remain the primary treatment modalities; however, limitations including postoperative recurrence and metastasis, chemotherapy resistance, and adverse effects persist [3,4]. Traditional Chinese medicine (TCM) has demonstrated unique advantages in comprehensive CRC management, particularly in improving quality of life, prolonging survival, and reversing multidrug resistance.

According to TCM theory, CRC pathogenesis is fundamentally characterized by "healthy Qi deficiency and pathogenic factors excess." As recorded in the Suwen · Linglan Secret Treatise: "The large intestine serves as the organ of conduction responsible for the conduction of waste." Dampness accumulation, Qi stagnation and blood stasis, together

with healthy Qi deficiency, constitute the core pathological mechanisms of CRC. Professor Yongheng He developed Tianma Granules (National Patent No. ZL201710036198.2) based on the "toxicity-deficiency causing disease" theory. The formula employs Scolopendra and Scorpio as sovereign drugs to attack toxins and dissipate masses, Lobelia chinensis and Phellodendron amurense as minister drugs to clear heat and remove toxins, and Astragalus membranaceus and Dioscorea opposita as assistant drugs to supplement Qi and strengthen the body. The complete prescription possesses effects of extracting cancer toxins, dissipating masses, unblocking meridians, and supplementing deficiencies [5]. Previous studies have confirmed that Tianma Granules can inhibit CRC cell proliferation through regulating the PI3K/Akt/mTOR signaling pathway and possess definite clinical efficacy [6-10].

PTEN, as a critical tumor suppressor gene, exerts inhibitory effects on tumor proliferation and induces apoptosis through negative regulation of the PI3K/AKT/mTOR signaling pathway [11,12]. Natural killer (NK) cells serve as the first line of defense in antitumor immunity, and NKG2D, as a crucial activating receptor on NK cells, directly influences NK cell cytotoxic activity [13,14]. Previous research has demonstrated that Tianma Granules can inhibit miR-21-5P expression, leading to elevated PTEN levels and subsequent suppression of the PI3K/AKT/mTOR pathway [15]. Based on the "toxicity-deficiency" theory, this study further explores whether modified Tianma Granules can induce HCT116 cell apoptosis through dual regulation of the PTEN/PI3K/AKT/mTOR signaling pathway and NKG2D expression, thereby providing experimental evidence for TCM treatment of CRC.

2. Materials and Methods

2.1. Experimental Animals and Materials

Thirty-two SPF-grade 4-week-old SD rats (half male and half female, weighing approximately 180 g) were purchased from [supplier] [License No: SCXK (Xiang) 2019-004]. Modified Tianma Granules consisted of Scolopendra 2 g, Scorpio 3 g, Lobelia chinensis 15 g, Phellodendron amurense 6 g, Sparganium stoloniferum 10 g, Rheum palmatum 6 g, Arisaema cum bile 3 g, Sargassum 10 g, Astragalus membranaceus 20 g, Dioscorea opposita 20 g, and Angelica sinensis 15 g (Guangdong Yifang Pharmaceutical Co., Ltd.). Human CRC cell line HCT116 and NK92 cells were obtained from [supplier].

Ethics Statement: All animal experiments were approved by the Animal Ethics Committee of [Institution] (Approval No. DW20240319-036) and conducted in accordance with the institutional guidelines for the care and use of laboratory animals.

2.2. Preparation of Drug-Containing Serum

The rats were randomly divided into four groups (n = 8 per group): the blank group (pure water by gavage), the detoxification group (Scolopendra, Scorpio, Lobelia chinensis, Phellodendron amurense, Sparganium stoloniferum, Rheum palmatum, Arisaema cum bile, and Sargassum by gavage), the tonifying deficiency group (Astragalus membranaceus, Dioscorea opposita, and Angelica sinensis by gavage), and the original prescription group (all components by gavage). After a one-week acclimation period, drugs were administered twice daily for one week at 10 times the equivalent human daily dose calculated for a 60-kg adult. Blood was collected from the abdominal aorta 2 h after the last administration. Serum was separated, inactivated at 56°C for 30 min, filter-sterilized through 0.22 μm membranes, and stored at −80°C. The working concentration of drug-containing serum was 20%.

2.3. Cell Culture and Drug Intervention

HCT116 cells were cultured in DMEM complete medium containing 10% fetal bovine serum, and NK92 cells were cultured in specific medium, both at 37°C with 5% CO_2. Cells in the logarithmic growth phase were seeded at 1×10^5 cells/mL and divided into five groups: Group A (blank control, complete medium), Group B (blank serum, 20% blank rat serum), Group C (original prescription, 20% original prescription serum), Group D (detoxification, 20% detoxification

serum), and Group E (tonifying deficiency, 20% tonifying deficiency serum). Each group had three replicates with 48 h of intervention.

2.4. Detection Methods

Cell apoptosis was detected by flow cytometry after Annexin V-FITC/PI double staining. NK cell activity was assessed by flow cytometric detection of CD56 and CD107a expression after co-culture of HCT116 and NK92 cells. For Western blot, total protein was extracted, protein concentration was determined by the BCA method, and PTEN and NKG2D protein expression levels were detected with GAPDH as the internal reference.

2.5. Statistical Analysis

SPSS 27.0 was used for statistical analysis. Measurement data were expressed as mean ± SD. One-way analysis of variance (ANOVA) was used for multi-group comparisons, followed by the LSD post-hoc test for pairwise comparisons. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Cell Morphological Observation

Under light microscopy, cells in the blank control and blank serum groups showed vigorous growth, covering the plate bottom with clear culture medium, distinct cell boundaries, and normal morphology. Cells in the detoxification, tonifying deficiency, and original prescription groups exhibited varying degrees of growth inhibition with turbid culture medium, floating dead cells, and blurred cell boundaries. The inhibitory effects were most pronounced in the original prescription and detoxification groups, while the tonifying deficiency group showed the weakest inhibition (**Figures 1–5**).

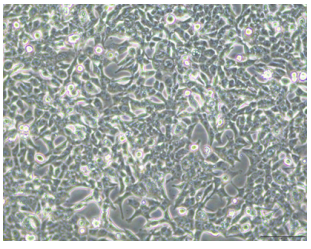

Figure 1. Blank control group

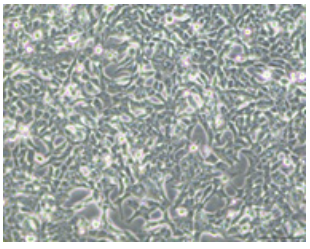

Figure 2. Blank serum group

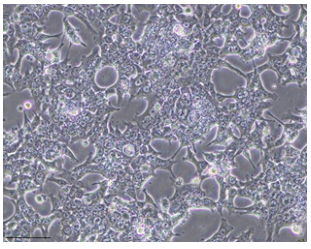

Figure 3. Detoxification group

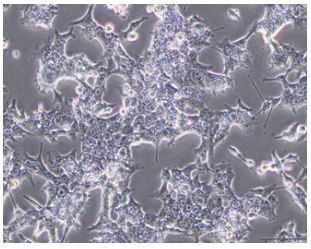

Figure 4. Tonifying deficiency group

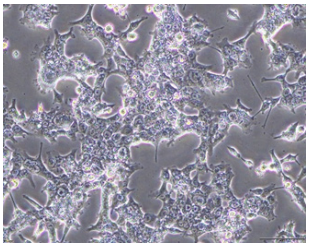

Figure 5. Original prescription group

3.2. Cell Apoptosis Rate

Flow cytometry results (**Table 1**) showed that the apoptosis rate in the original prescription group (11.60% ± 1.40%) was significantly higher than that in the blank control group (1.70%±0.78%) and blank serum group (2.01% ± 0.04%) ($P < 0.05$). The apoptosis rates in the detoxification group (6.80% ± 0.15%) and tonifying deficiency group (3.38% ± 0.31%) were also significantly higher than those in the blank control group ($P < 0.05$). The original prescription group showed a significantly higher apoptosis rate than the detoxification and tonifying deficiency groups ($P < 0.05$).

Table 1. Comparison of cell apoptosis rates among groups (mean ± SD, n = 3)

Group	Apoptosis rate (%)
Blank control	1.70 ± 0.78
Blank serum	2.01 ± 0.04
Original prescription	11.60 ± 1.40*†
Detoxification	6.80 ± 0.15*
Tonifying deficiency	3.38 ± 0.31*

Note: *P < 0.05 vs. blank control group; †P < 0.05 vs. detoxification and tonifying deficiency groups.

3.3. NK Cell Activity Marker Expression

Flow cytometry results (**Table 2**) showed that CD56 expression (7.49% ± 0.74%) and CD107a expression (22.04% ± 1.57%) in the original prescription group were significantly higher than those in the blank control group (1.32% ± 0.33% and 5.48% ± 0.42%) (P < 0.05). CD56 (5.22% ± 0.34%) and CD107a (15.56% ± 0.49%) expression in the detoxification group were also significantly higher than those in the blank control group (P < 0.05). The original prescription group showed significantly higher CD56 and CD107a expression than the detoxification and tonifying deficiency groups (P < 0.05).

Table 2. Comparison of CD56 and CD107a expression among groups (mean ± SD, n = 3)

Group	CD56 (%)	CD107a (%)
Blank control	1.32 ± 0.33	5.48 ± 0.42
Blank serum	1.16 ± 0.16	8.67 ± 0.30
Original prescription	7.49 ± 0.74*†	22.04 ± 1.57*†
Detoxification	5.22 ± 0.34*	15.56 ± 0.49*
Tonifying deficiency	3.53 ± 0.26*	9.69 ± 0.08*

*Note: *P < 0.05 vs. blank control group; †P < 0.05 vs. detoxification and tonifying deficiency groups.*

3.4. PTEN and NKG2D Protein Expression

Western blot results (**Table 3**) showed that PTEN expression (0.86 ± 0.11) and NKG2D expression (0.64 ± 0.08) in the original prescription group were significantly higher than those in the blank control group (0.42 ± 0.05 and 0.39 ± 0.04) (P < 0.05). The detoxification group showed the highest PTEN expression (0.88 ± 0.12), while the tonifying deficiency group showed the highest NKG2D expression (0.87 ± 0.11), both significantly higher than the blank control group (P < 0.05). The original prescription group showed intermediate PTEN and NKG2D expression levels between the two component groups (**Figure 6**).

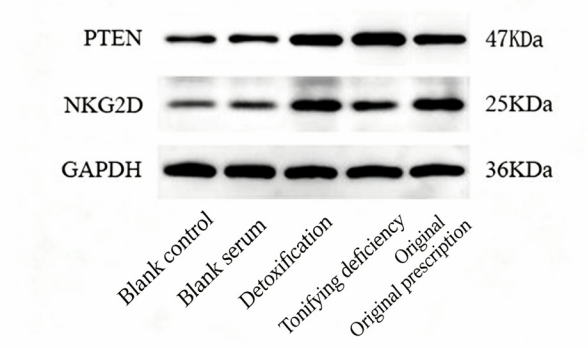

Figure 6. Western blot results

Table 3. Comparison of PTEN and NKG2D protein expression among groups (mean ± SD, n = 3)

Group	PTEN/GAPDH	NKG2D/GAPDH
Blank control	0.42 ± 0.05	0.39 ± 0.04
Blank serum	0.49 ± 0.06	0.44 ± 0.06
Original prescription	0.86 ± 0.11*	0.64 ± 0.08*
Detoxification	0.88 ± 0.12*	0.76 ± 0.10*
Tonifying deficiency	0.64 ± 0.09*	0.87 ± 0.11*

Note: *$P < 0.05$ vs. blank control group.

4. Discussion

This study, based on the TCM "toxicity-deficiency" theory, investigated the mechanism of modified Tianma Granules in inducing apoptosis in CRC HCT116 cells through in vitro experiments. The results demonstrate that mTMG can inhibit the PI3K/AKT/mTOR signaling pathway by up-regulating PTEN expression while simultaneously enhancing NKG2D expression to activate NK cell immune function, thereby exerting synergistic antitumor effects through "detoxification and pathogen elimination" combined with "strengthening the body and consolidating the foundation."

PTEN dephosphorylates PIP3 to PIP2 through its lipid phosphatase activity, thereby negatively regulating the PI3K/AKT/mTOR pathway and inhibiting tumor cell proliferation and inducing apoptosis [11,12]. In this study, PTEN expression was significantly up-regulated in both the original prescription and detoxification groups (0.86 ± 0.11 and 0.88 ± 0.12, respectively), suggesting that the toxin-attacking drugs (Scolopendra and Scorpio) are the primary active components responsible for PTEN up-regulation. The slightly higher PTEN elevation in the detoxification group compared to the original prescription group may be attributed to multi-target regulatory effects of the tonifying drugs on the overall signaling network. This finding is consistent with previous studies demonstrating that Tianma Granules block the PI3K/AKT/mTOR pathway by inhibiting miR-21-5P expression and increasing PTEN levels [15].

NKG2D is the most important activating receptor on NK cells, and its binding to NKG2D ligands on tumor cell surfaces activates NK cell cytotoxic effects [13,14]. CD107a serves as a marker of NK cell degranulation, while CD56 is a phenotypic marker of NK cells; elevated expression of both reflects enhanced NK cell killing activity [16-18]. In this study, NKG2D expression was significantly increased in the original prescription and tonifying deficiency groups (0.64 ± 0.08 and 0.87 ± 0.11, respectively), with the tonifying deficiency group showing effects superior to those of the detoxification group. The results for CD56 and CD107a further confirmed significantly enhanced NK cell killing activity in the original prescription and detoxification groups. These findings indicate that tonifying drugs such as Astragalus membranaceus, Dioscorea opposita, and Angelica sinensis play a crucial role in enhancing NK cell immune function, while detoxification drugs increase tumor cell sensitivity to NK cell-mediated killing through direct inhibition of tumor cells.

Through formula decomposition, this study further elucidated the functional characteristics of mTMG: the detoxification component primarily exerts direct tumor inhibition (PTEN up-regulation, apoptosis rate 6.80%), the tonifying deficiency component predominantly mediates immune regulation (NKG2D up-regulation), and the original prescription component combines both advantages (apoptosis rate 11.60%, with significantly elevated PTEN and NKG2D). This embodies the TCM principle of "attacking and supplementing simultaneously" [19].

This study has several limitations. This study used only the HCT116 cell line and lacked in vivo animal model validation. Detection of downstream key molecules in the PI3K/AKT/mTOR pathway was not comprehensive, and the upstream regulatory mechanism of NKG2D requires further exploration. Future studies should establish xenograft tumor animal models and conduct multicenter clinical studies to further clarify the clinical application value of modified Tianma Granules.

5. Conclusion

Modified Tianma Granules can induce HCT116 cell apoptosis through dual mechanisms: up-regulating PTEN expression to suppress activation of the PI3K/AKT/mTOR signaling pathway, and enhancing NKG2D expression to activate NK cell immune killing function. The detoxification and tonifying deficiency components exhibit clear synergistic effects, providing experimental evidence for the TCM "attacking and supplementing simultaneously" approach in CRC treatment.

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Disclosure statement

The author declares no conflict of interest.

References

[1] Baidoun F, Elshiwy K, Elkeraie Y, et al., 2021, Colorectal cancer epidemiology: recent trends and impact on outcomes. Curr Drug Targets, 22(9): 998-1009.

[2] Hossain MS, Karuniawati H, Jairoun AA, et al., 2022, Colorectal cancer: a review of carcinogenesis, global epidemiology, current challenges, risk factors, preventive and treatment strategies. Cancers, 14(7): 1732.

[3] Zhou Y, Zhang WB, Hu J, et al., 2025, Clinical efficacy of cetuximab combined with FOLFIRI in advanced colon cancer. Chin J Clin Rational Drug Use, 18(5): 103-106.

[4] Wang ZC, Yang GD, Chen QY, et al., 2024, Evaluation of therapeutic effect of laparoscopic colon cancer resection. Chin J Mod Drug Appl, 18(24): 14-17.

[5] Zhang T, Ding N, Luo HB, et al., 2022, Professor Yongheng He's experience in treating colorectal cancer from "toxicity and deficiency." J Hunan Univ Chin Med, 42(6): 1023-1028.

[6] Zhong YC, 2010, Toxicological experimental study of Tianma Granules, Hunan University of Chinese Medicine.

[7] Huang GD, Li JB, He YH, et al., 2006, Clinical study of Tianma Granules in treating advanced colorectal cancer patients unsuitable for radical surgery. Chin J Emerg Tradit Chin Med, (12): 1346-1347.

[8] Huang CR, Guo SM, He YH, 2004, Clinical study of Tianma Granules on prolonging survival after radical colorectal cancer surgery. J Tongji Univ (Med Sci), (1): 58-60.

[9] Peng TS, Xie B, Nie T, et al., 2016, Clinical efficacy of Tianma Granules in inhibiting postoperative recurrence of colorectal cancer. Chin J Pharmaco Clin, 32(3): 191-193.

[10] Tang LJ, Huang GD, Huang YH, et al., 2007, Clinical study of Tianma Granules perfusion in treating low rectal cancer. Chin J Emerg Tradit Chin Med, (11): 1344-1346.

[11] Qi SS, Yang L, Lu WJ, et al., 2020, Research progress in regulating PTEN expression and activity. Chin J Pathophysiol, 36(12): 2289-2293.

[12] Fan YX, Abulimiti P, Zhang HL, et al., 2017, Mechanism of reversal of multidrug resistance by curcumin in human colorectal cancer cell line HCT-8/5-FU. Genet Mol Res, 16(2).

[13] Tang F, Li J, Qi L, et al., 2023, A pan-cancer single-cell panorama of human natural killer cells. Cell, 186(19): 4235-4251.

[14] Dhar P, Wu JD, 2018, NKG2D and its ligands in cancer. Curr Opin Immunol, 51: 55-61.

[15] Deng SH, 2020, Formula decomposition study of Tianma Granules and the effect of its simplified formula on PI3K/Akt/mTOR signaling pathway in colorectal cancer, Hunan University of Chinese Medicine.
[16] Cohnen A, et al., 2013, Surface CD107a/LAMP-1 protects natural killer cells from degranulation-associated damage. Blood, 122(8): 1411-1418.
[17] Aktas E, et al., 2009, Relationship between CD107a expression and cytotoxic activity. Cell Immunol, 254(2): 149-154.
[18] Yoshida M, et al., 2015, Neural cell adhesion molecule (CD56) in health and disease. J Appl Hematol, 6: 93-105.
[19] Luo WJ, Guo JY, Zhao TF, et al., 2023, Li Yi's experience in treating lung cancer from "toxicity and deficiency." Clin J Chin Med, 35(9): 1718-1722.