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The Effect of Curcumin on TGF-β-Induced Proliferation, Migration, and Invasion of Gastric Cancer AGS Cells

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Abstract: *This paper aims to investigate the effects of curcumin on TGF-β-induced proliferation, migration, and invasion of gastric cancer AGS cells and preliminarily explore its cancer-suppressing pathways. Curcumin and TGF-β were used to treat AGS cells cultured in vitro. Cell proliferation, migration, and invasion capabilities were assessed by MTT assay, scratch assay, and Transwell assay, respectively. Western blot was used to detect the expression levels of E-cadherin, Vimentin, Bcl-2, Bax, and signaling pathway proteins Akt, P-Akt, and FASN. RT-PCR was employed to determine FASN mRNA expression. Curcumin counteracted the effects of TGF-β on the proliferation, migration, and invasion of AGS cells. In the TGF-β group, E-cadherin expression was downregulated, while Vimentin, P-Akt, and FASN expression were upregulated. In the curcumin + TGF-β group, E-cadherin and Bax were upregulated, whereas Vimentin, Bcl-2, P-Akt, and FASN were downregulated compared to the TGF-β group. Curcumin antagonized the TGF-β-induced upregulation of FASN-mRNA expression in AGS cells.Curcumin may counteract TGF-β-induced proliferation, migration, and invasion of gastric cancer AGS cells by reducing Akt phosphorylation and inhibiting FASN synthesis, thus altering the synthetic metabolism of the cells and inhibiting their epithelial-to-mesenchymal transition.*

Keywords: *Gastric cancer, curcumin, transforming growth factor-β, fatty acid synthetase*

1. Introduction

Transforming growth factor-β (TGF-β) expression is significantly associated with the prognosis of gastric cancer. Secreted by various cells, TGF-β promotes angiogenesis in the tumor microenvironment while suppressing the immune system and can induce epithelial-to-mesenchymal transition (EMT), which is a key process in tumorigenesis. EMT is characterized by changes in key biomarkers (E-cadherin, Vimentin, and N-cadherin) leading to decreased cell adhesion, loss of polarity, and reduced tight junctions. The PI3K-Akt-mTOR signaling pathway is one of the primary EMT pathways mediated by TGF-β and regulates the synthesis of fatty acid synthetase (FASN).[1]

Curcumin has shown potential in treating various diseases through the modulation of multiple signaling pathways, including wound healing, urinary tract infections, and gastrointestinal diseases such as gastric cancer. Compared to chemotherapeutic drugs, curcumin is less toxic and more biocompatible, offering promising applications in cancer therapy. Therefore, in this study, we employed TGF-β-induced gastric cancer AGS cells to observe curcumin's effects on the proliferation, migration, and invasion of these cells and to preliminarily investigate the molecular mechanisms by assessing the expression of FASN, Akt, and P-Akt, through which curcumin inhibits TGF-β-induced migration and invasion in gastric cancer AGS cells.

2. Materials and Methods

2.1 Cell Source and Main Reagents

The human gastric cancer AGS cell line was obtained from the National Cell Resource Sharing Platform. DMEM medium was purchased from HyClone; 0.25% Trypsin-EDTA and PBS were obtained from Shanghai Beyotime Biotechnology. Fetal bovine serum (FBS) was purchased from GIBCO, USA. Antibodies for FASN, E-cadherin, Vimentin, P-Akt, Bax, Bcl-2, and GAPDH were

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purchased from Proteintech, while the Akt antibody was acquired from Abcam. An apoptosis assay kit was provided by Dalian Biotechnology Co., Ltd., and Real-time PCR kits were obtained from TransGen Biotech, with a reverse transcription kit purchased from TIANGEN Biotech Co., Ltd. (Beijing, China).[2-5]

2.2 Cell Culture, Transfection, and Grouping

Human gastric cancer AGS cells were cultured in DMEM supplemented with 10% FBS in an incubator at 37°C with 5% CO_2. Previous studies have extensively reported curcumin's inhibitory effects on various gastric cancer cell lines, so this study does not replicate these experiments. The experimental groups were divided into Curcumin + TGF-β group (10 μmol/L curcumin + 5 ng/ml TGF-β), TGF-β group (5 ng/ml TGF-β), and Control group (equal volume of buffer solution).

2.3 MTT Assay for Cell Proliferation in Each Group

Cells in the logarithmic growth phase were digested with 0.25% trypsin, resuspended, and counted, with 3,000 cells per well seeded in 96-well plates. After cell adhesion, the supernatant was gently removed, and each well was treated with either 10 μmol/L curcumin + 5 ng/ml TGF-β, 5 ng/ml TGF-β, or an equal volume of buffer solution (control group), with three replicates per group. After 24, 48, 72, and 96 hours of incubation, 100 μL of 10% MTT solution was added to each well and incubated for 4 hours. Following incubation, the supernatant was discarded, 150 μL of DMSO was added, and the plates were shaken for 10 minutes. Absorbance was measured at 595 nm using a microplate reader.

2.4 Scratch Assay for Cell Migration

Cells in the logarithmic growth phase were seeded at 8×10^5 cells per well in six-well plates and incubated at 37°C with 5% CO_2 for 24 hours. Upon reaching near-confluence, a scratch was made in the center of each well. The experimental groups were treated with 10 μmol/L curcumin + 5 ng/ml TGF-β, 5 ng/ml TGF-β, or an equal volume of buffer solution, with three replicates per group. Cell migration was observed under an inverted microscope at 0 and 48 hours, and images were analyzed for migration area using Image J software.

2.5 Transwell Assay for Cell Invasion

Cells in the logarithmic growth phase were adjusted to a concentration of 1×10^5 cells/mL. Approximately 100 μL of cell suspension with treatment was added to the upper chamber of a Transwell insert placed in a 24-well plate, with 200 μL medium containing treatment and 800 μL serum-containing medium in the lower chamber. The assay was incubated at 37°C with 5% CO_2 for 24 hours. Cells were then fixed in 4% paraformaldehyde at room temperature for 30 minutes, stained with 2% crystal violet for 30 minutes, and rinsed twice with PBS. Non-migrated cells on the upper chamber membrane were gently removed, air-dried, and counted under a microscope, with images captured for analysis.

2.6 Western Blotting for Protein Expression

Proteins were extracted from each group after 48 hours of treatment, and concentrations were measured. Samples were loaded into gels with 6 μL marker and 10 μL of protein, followed by electrophoresis, membrane transfer, and blocking. After incubation with primary and secondary antibodies, detection was performed by color development and imaging.[6-10]

2.7 qRT-PCR for FASN Expression in Each Group

Table 1 Different primer sequences

Primers	Sequences
Human ACTB _F	*CATGTACGTTGCTATCCAGGC*
Human ACTB _R	*CTCCTTAATGTCACGCACGAT*
homo FAS_F	*AAGGACCTGTCTAGGTTTGATGC*
homo FAS_R	*TGGCTTCATAGGTGACTTCCA*

After 48 hours of treatment, RNA was extracted from each group and reverse-transcribed to

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synthesize cDNA for PCR analysis. The specific primer sequences are listed in the table 1.

2.8 Statistical Methods

Statistical analyses were performed using SPSS 21.0 and GraphPad Prism 8.0. Data are presented as mean ± standard deviation ($\bar{x} \pm s$). One-way ANOVA was used for comparisons among multiple groups, and independent sample t-tests were used for comparisons between two groups. All experiments were repeated three times, with $P < 0.05$ indicating statistical significance.

3. Results

3.1 Effect of Curcumin on TGF-β-Induced Proliferation of Gastric Cancer AGS Cells

As shown in Table 2 and Figure 1, over time, the absorbance of the TGF-β group significantly increased compared to the control group, with a statistically significant difference between the two groups ($P < 0.01$). In contrast, the absorbance of the Curcumin + TGF-β group significantly decreased compared to the TGF-β group, with a statistically significant difference between the two groups ($P < 0.05$), except at 48 hours, where the difference was not statistically significant.

Table 2 Changes in the absorbance of the cells at different times in each group

group	24h	48h	72h	96h
control	0.614±0.012	0.862±0.010	1.116±0.010	1.465±0.023
TGF-β	0.659±0.010**	1.072±0.090**	1.464±0.045**	1.850±0.028**
curcumin + TGF-β	0.628±0.003#	0.999±0.023	1.276±0.039##	1.628±0.058##

Note: ** P<0.01 when compared with the control group.
##P<0.01 between experimental groups. #P<0.05 between experimental groups.

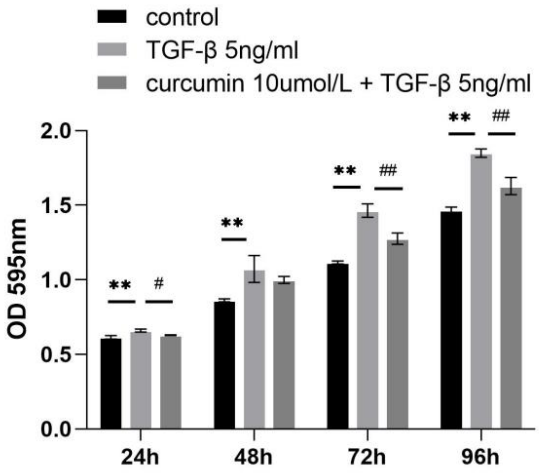

Figure 1 Changes of control, TGF-β and curcumin + TGF-β on viability of AGS cells in 24, 48, 72, and 96 hours.

Note: ** P<0.01 when compared with the control group.
##P<0.01 between experimental groups. #P<0.05 between experimental groups.

3.2 Effect of Curcumin on TGF-β-Induced Migration Ability of Gastric Cancer AGS Cells

Table 3 Mobility of the cells in each group

group	48h(%)
control	34.75±3.79
TGF-β	59.51±2.87**
curcumin + TGF-β	46.74±3.98##

Note: ** P<0.01 when compared with the control group. ##P<0.01 between experimental groups.

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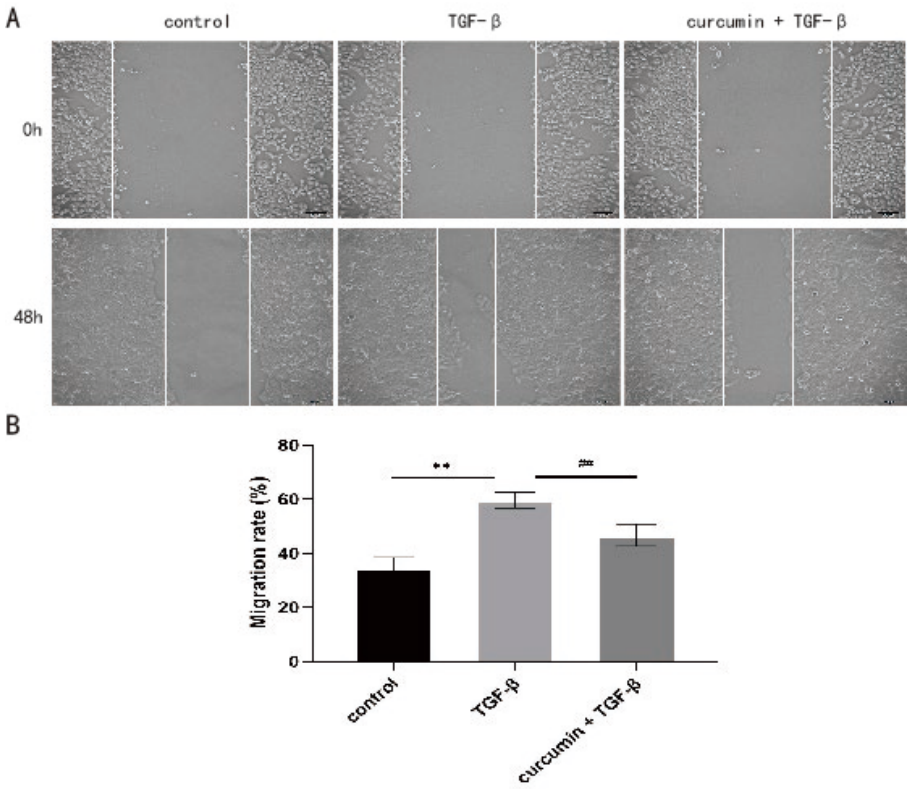

Figure 2 Mobility of the cells in each group. The effect of curcumin + TGF-β and TGF-β groups on gastric cancer cell migration under white light (magnification 200x) (A). Bar plot of the effects of curcumin + TGF-β and TGF-β groups on gastric cancer cell migration (B).

** P<0.01 when compared with the control group. ##P<0.01 between experimental groups.

As shown in Table 3 and Figure 2, the scratch wound healing area in the TGF-β group increased significantly compared to the control group, with a statistically significant difference between the two groups ($P < 0.01$). In contrast, the wound healing area in the Curcumin + TGF-β group decreased significantly compared to the TGF-β group, with a statistically significant difference ($P < 0.01$).

3.3 Effect of Curcumin on TGF-β-Induced Invasive Ability of Gastric Cancer AGS Cells

As shown in Table 4 and Figure 3, the number of transmembrane cells significantly increased after TGF-β treatment compared to the control group, with a statistically significant difference ($P < 0.01$). In contrast, the Curcumin + TGF-β group exhibited a reduced number of transmembrane cells compared to the TGF-β group, also showing a statistically significant difference ($P < 0.01$).

Table 4 The number of invaded cells in each group

group	number of invaded cells (/ field)
control	42±6
TGF-β	80±8**
curcumin + TGF-β	60±5##

Note: ** P<0.01 when compared with the control group. ##P<0.01 between experimental groups.

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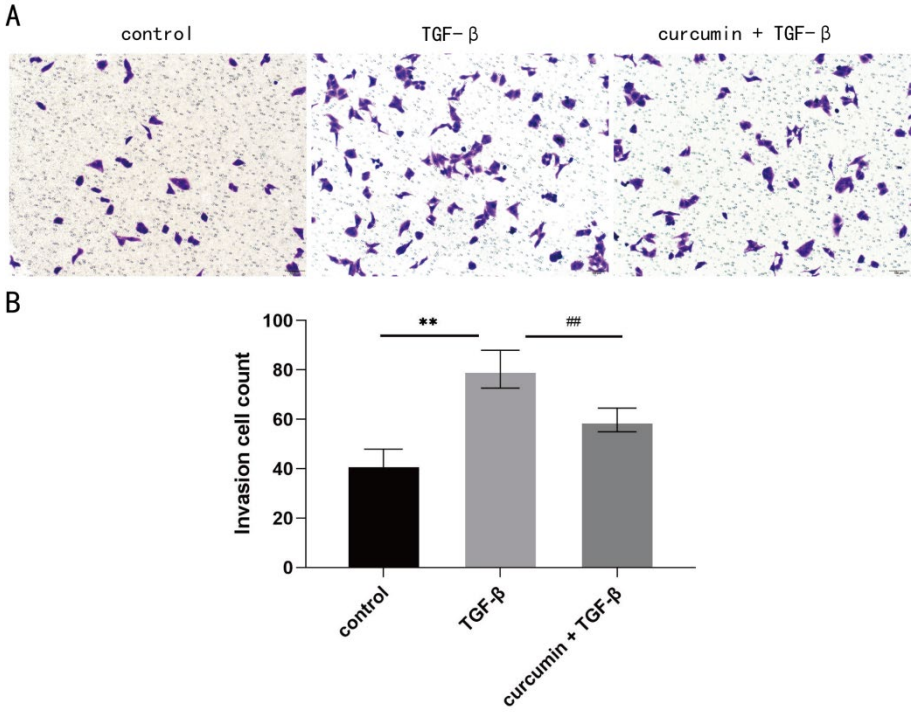

Figure 3 The number of invaded cells in each group. The effect of curcumin + TGF-β and TGF-β groups on gastric cancer cell invasion under white light (magnification 200x) (A). Bar plot of the effects of curcumin + TGF-β and TGF-β groups on gastric cancer cell invasion (B).

** P<0.01 when compared with the control group. ##P<0.01 between experimental groups.

3.4 Western Blot Analysis of Relevant Protein Expression

3.4.1 Effect of Curcumin + TGF-β and TGF-β on the Expression of Epithelial to Mesenchymal Transition (EMT) Related Proteins E-cadherin and Vimentin

As shown in Figure 4 below, treatment of gastric cancer AGS cells with TGF-β reduced the expression level of E-cadherin compared to the control group, with a statistically significant difference ($P < 0.01$). In the Curcumin + TGF-β group, E-cadherin expression increased compared to the TGF-β group, with a statistically significant difference ($P < 0.05$). Vimentin expression in the TGF-β group was higher than in the control group, while it was reduced in the Curcumin + TGF-β group compared to the TGF-β group, both showing statistically significant differences ($P < 0.01$ and $P < 0.05$, respectively).

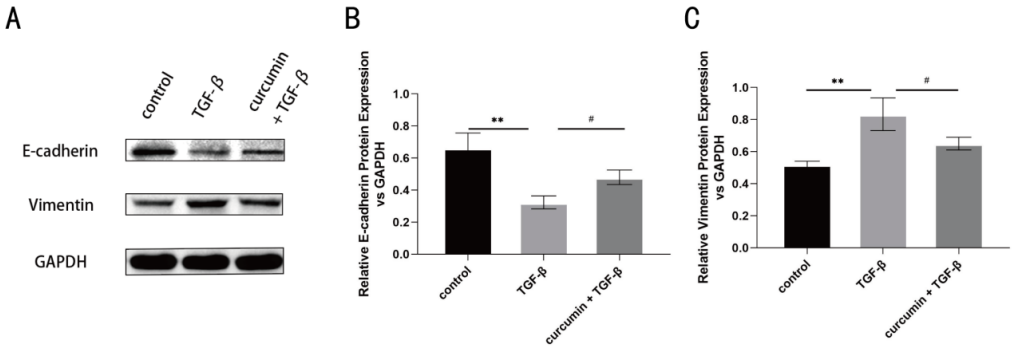

Figure 4 Western blot to analyze the effect of curcumin and TGF-β on the expression level of EMT-related protein in gastric cancer AGS cells. EMT-related protein expression in different groups of cells (A). Bar graph of the E-cadherin expression in each group (B). Bar plot of Vimentin expression in each group (C).

** P<0.01 when compared with the control group. * P<0.05 when compared with the control group. ##P<0.01 between experimental groups. #P<0.05 between experimental groups.

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3.4.2 Effect of Curcumin + TGF-β and TGF-β on the Expression of Apoptosis-Related Proteins Bax and Bcl-2

As shown in the figure 5, there was no statistically significant difference in the expression level of the anti-apoptotic factor Bcl-2 between the TGF-β group and the control group. However, Bcl-2 expression in the Curcumin + TGF-β group was significantly lower than in both the TGF-β group and the control group, with statistical significance ($P < 0.01$). For the pro-apoptotic factor Bax, no statistically significant difference was observed between the TGF-β group and the control group, but the expression level in the Curcumin + TGF-β group was significantly higher than in both the TGF-β group and the control group, with statistical significance ($P < 0.01$).

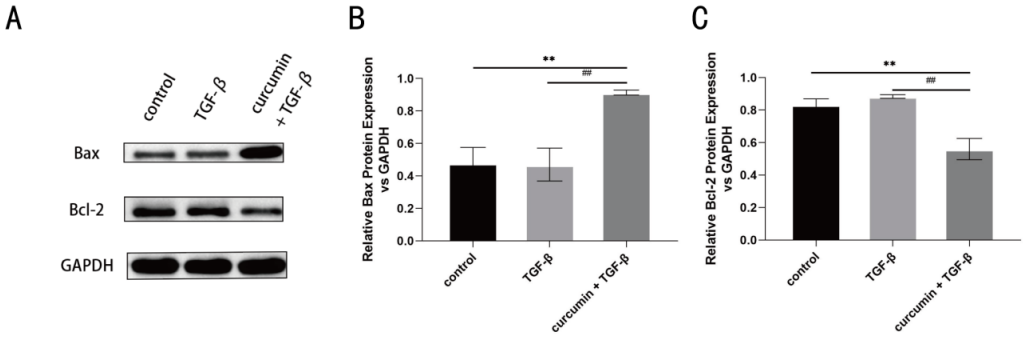

Figure 5 The effects of curcumin and TGF-β on protein expression of apoptosis in gastric cancer AGS cells. Apoptosis-related protein expression in different groups of cells (A). Bar graph of the Bax expression in each group (B). Bar plot of Bcl-2 expression in each group (C).

** P<0.01 when compared with the control group. * P<0.05 when compared with the control group. ##P<0.01 between experimental groups. #P<0.05 between experimental groups.

3.4.3 Effect of Curcumin + TGF-β and TGF-β on the Expression of Akt, P-Akt, and FASN

As shown in the figure 6, FASN levels were significantly higher in the TGF-β group compared to the control group ($P < 0.01$), while in the Curcumin + TGF-β group, FASN expression was significantly reduced compared to the TGF-β group ($P < 0.05$). Findings indicated no significant differences in Akt expression across groups. However, P-Akt levels were elevated in the TGF-β group relative to the control group, with statistical significance ($P < 0.05$), whereas in the Curcumin + TGF-β group, P-Akt levels were significantly reduced compared to the TGF-β group ($P < 0.05$).

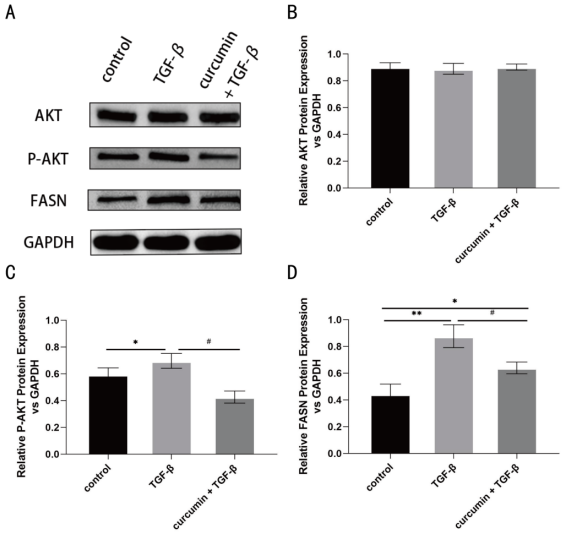

Figure 6 The effects of curcumin and TGF-β on the expression level of each protein in gastric cancer cells. Expression of each protein in different groups of cells (A). Bar graph of the AKT expression in each group (B). Bar plot of P-AKT expression in each group (C). Bar plot of FASN expression in each group (D).

** P<0.01 when compared with the control group. * P<0.05 when compared with the control group. ##P<0.01 between experimental groups. #P<0.05 between experimental groups.

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3.4.4 Effect of Curcumin + TGF-β and TGF-β on FASN mRNA Expression Levels

As shown in the figure 7, FASN mRNA levels were significantly elevated in the TGF-β group compared to the control group ($P < 0.01$), while the Curcumin + TGF-β group showed a significant reduction in FASN mRNA levels compared to the TGF-β group ($P < 0.01$).

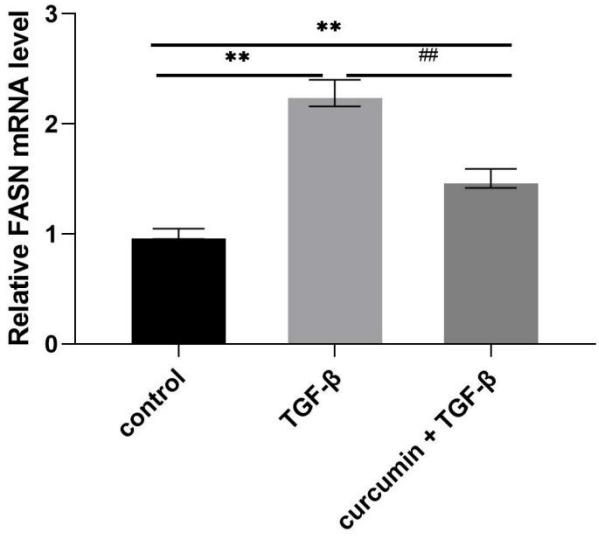

Figure 7 The mRNA expression of FASN in each group was quantified by quantitative fluorescence PCR.

P<0.01 when compared with the control group. ##P<0.01 between experimental groups.

4. Discussion

Transforming growth factor-β (TGF-β) regulates cell cycle progression, apoptosis, adhesion, and differentiation. Tumor cells can selectively evade TGF-β's inhibitory functions, utilizing its promoting effects to gain a growth advantage and induce epithelial-mesenchymal transition (EMT), thereby enhancing their proliferation, migration, and invasion abilities. EMT describes the process where epithelial cells lose their characteristics and acquire mesenchymal traits. Changes in epithelial marker E-cadherin and mesenchymal marker Vimentin expression levels are critical and indicative of EMT. This study's cellular experiments further confirmed that TGF-β could induce EMT in human gastric cancer AGS cells, enhancing their proliferation, migration, and invasion capacities, simulating TGF-β's induction effect in vivo on tumor cells. Western blot experiments demonstrated decreased expression of E-cadherin and increased Vimentin expression following TGF-β treatment, substantiating TGF-β's role in promoting EMT in gastric cancer AGS cells. Through curcumin-treated groups, it was observed that curcumin effectively counteracted TGF-β-induced EMT, significantly reducing the proliferative, migratory, and invasive capabilities of AGS cells.

Several common oncogenes activate the Akt/mTOR pathway, increasing regulatory factors that promote the expression of fatty acid synthase (FASN) mRNA. The intensity of FASN expression is directly related to tumor stage and malignancy, indicating that FASN not only provides metabolic advantages for cancer cell survival and proliferation but also promotes a more aggressive phenotype. This study found that P-Akt and FASN levels were significantly elevated in the TGF-β group compared to the control group, while both P-Akt and FASN levels significantly decreased in the curcumin-treated group relative to the TGF-β group. TGF-β appears to promote the EMT process and FASN synthesis via Akt phosphorylation, while curcumin effectively counteracts this phosphorylation process, thus inhibiting FASN synthesis and curtailing TGF-β-induced migration and invasion capabilities.[11,12]

An imbalance between pro-apoptotic and anti-apoptotic factors, triggered by either internal abnormalities or external stimuli, may lead to tumorigenesis if the body cannot compensate. Bax promotes apoptosis through the intrinsic pathway and is linked to cancer development and prognosis. Western blot analysis of apoptosis-related proteins Bcl-2 and Bax revealed that the expression level of the anti-apoptotic factor Bcl-2 in the curcumin intervention group was significantly lower than in the

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other two groups, while the pro-apoptotic factor Bax was significantly higher than in the other groups, suggesting that curcumin may induce tumor cell apoptosis through the intrinsic pathway.

In summary, curcumin significantly counteracts TGF-β-induced EMT in gastric cancer AGS cells, potentially by reducing Akt phosphorylation and inhibiting FASN synthesis. This, in turn, alters cellular metabolism to reduce tumor cell proliferation, migration, and invasion capabilities.[13]

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