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*Original Article*

# TRAIL affects NF-κB and Survivin expressions in gastric cancer cell

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**Abstract:** Gastric cancer is a common malignancy in digestive tract. Tumor necrosis factor related apoptosis-inducing ligand (TRAIL) can selectively induce tumor cell apoptosis. Survivin and NF-κB participate in the cell apoptosis process induced by TRAIL. This study investigated the impact of TRAIL on NF-κB and Survivin expressions in gastric cancer cell and related mechanism. Gastric cancer cell line SGC-7901 was treated by TRAIL at 100 μg/L, 200 μg/L, and 500 μg/L for 12 h, 24 h, and 48 h, respectively. Cell proliferation was tested by MTT assay. Cell apoptosis was evaluated by flow cytometry. Cell invasion and migration were determined by Transwell assay. Survivin and NF-κB expressions were detected by Western blot. TRAIL significantly inhibited cell proliferation at different concentrations and times compared with control ($P < 0.05$). TRAIL intervention at 500 μg/L for 48 h exhibited the strongest suppressive effect on cell proliferation. It obviously promoted cell apoptosis and declined cell invasion and migration ($P < 0.05$). Survivin and NF-κB protein expressions markedly down regulated in SGC-7901 cells treated by TRAIL with time and dose dependence ($P < 0.05$). TRAIL significantly inhibited SGC-77901 cell proliferation, facilitated cell apoptosis, and restrained cell invasion and migration through suppressing Survivin and NF-κB.

**Keywords:** TRAIL, gastric cancer, NF-κB, Survivin

## Introduction

Gastric cancer is a common malignant tumor of the digestive system. Tumor cell apoptosis plays an important role in the occurrence and development processes of cancer. It is mainly presented as the inactivation of apoptosis-inducing gene and overexpression of apoptosis inhibiting gene. Blockage of cell apoptosis appears in precancerous lesions. Some molecules participate in the signaling transduction to affect apoptosis [1-4]. Tumor necrosis factor related apoptosis inducing ligand (TRAIL) is a member of the tumor necrosis factor family that can selectively induce tumor cell apoptosis without significant cytotoxicity [5]. It was showed that TRAIL is not sensitive to all the malignant cells. Survivin and NF-κB are the critical factors in cell apoptosis pathway induced by TRAIL [6]. Survivin, a new member of the apoptosis inhibiting protein family, is the strongest apoptosis inhibiting factor so far. It was found that Survivin has dual effect on cell behavior, including apoptosis inhibition and proliferation regulation. It plays inhibiting effect through directly suppressing Caspase-3 and Caspase-7, which is specifically expressed in a variety of tumors [7]. NF-κB is a kind of transcription factor existed in eukaryocytes with multiple biological functions. It regulates various genes expressions, such as cytokines, adhesion molecules, and growth factors. Its relationship with tumor attracts attention because of its role as a pleiotropic regulatory protein [8]. P65 is the major component of the active NF-κB. It plays a central role in tumor cell apoptosis and proliferation as a multifunction transcription factor. Its aberrant transcription is closely associated with the up regulation of anti-apoptotic genes. It was demonstrated that NF-κB plays a critical role in multiple malignant tumors, such as leukemia, lymphoma, and solid tumors. It is still controversy about the role of Survivin and NF-κB in the occurrence and development of gastric cancer. This study used TRAIL to treat gastric cancer cell line SGC-7901 to analyze its impact on Survivin and NF-κB expressions and related mechanism.

**Table 1.** TRAIL affected SGC-7901 cell proliferation (OD value)

| Time | Experimental group | | | Control |
|---|---|---|---|---|
| | 100 μg/L | 200 μg/L | 500 μg/L | |
| 12 h | 0.985±0.072[1] | 0.723±0.046[1,2] | 0.232±0.024[1,2,3] | 0.071±0.021 |
| 24 h | 0.811±0.045[1,4] | 0.512±0.034[1,2,4] | 0.176±0.016[1,2,3,4] | 0.072±0.017 |
| 48 h | 0.321±0.031[1,4,5] | 0.256±0.026[1,2,4,5] | 0.121±0.011[1,2,3,4,5] | 0.067±0.019 |

[1]P < 0.05, compared with control; [2]P < 0.05, compared with 100 μg/L; [3]P < 0.05, compared with 200 μg/L; [4]P < 0.05, compared with 12 h; [5]P < 0.05, compared with 24 h.

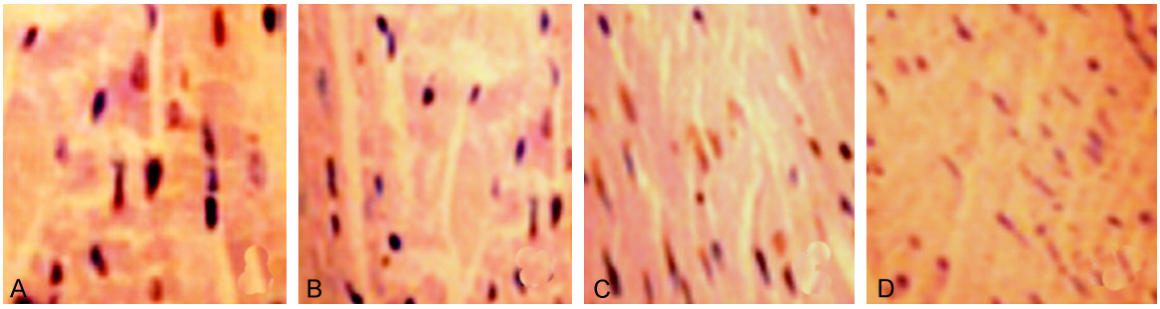


**Figure 1.** TRAIL affected SGC-7901 cell apoptosis (×400). A. 100 μg/L; B. 200 μg/L; C. 500 μg/L; D. Control.

## Materials and methods

### *Experimental cells*

Gastric cancer cell line SGC-7901 was provided by Soochow University (Suzhou, China).

### *Reagents*

TRAIL (Sinopharm Chemical Reagent co., Ltd, lot no. 20050913). TUNEL assay kit (Roche, lot no. 11684817910). Rabbit anti human Survivin and NF-κB polyclonal antibodies, and goat anti rabbit secondary antibody (Santa Cruz). RPMI 1640 medium, penicillin-streptomycin, FBS (Gibco).

### *Instruments*

Incubator (Thermo). Carbon dioxide incubator (SANYO). Inverted microscope (Nikon). Desktop low temperature centrifuge (Beckman). -80°C refrigerator (SANYO).

## Methods

### *Routine cell culture*

Gastric cancer cell line SGC-7901 was maintained in RPMI 1640 medium and cultured in incubator at 37°C and 5% $CO_2$.

### *TRAIL intervention*

SGC-7901 cells in logarithmic phase were seeded in the plate overnight. After cultured in 2% FBS for 24 h, the cells were maintained in RPMI 1640 medium containing 10% FBS and treated by TRAIL at 100 μg/L, 200 μg/L, and 500 μg/L for 12 h, 24 h, and 48 h, respectively. Untreated SGC-7901 cells were selected as normal control.

### *MTT assay*

The cells were treated by TRAIL at 100 μg/L, 200 μg/L, and 500 μg/L for 12 h, 24 h, and 48 h, respectively. The cells were added with 20 μl MTT solution at 5 mg/ml for 4 h. Then the cells were added with 150 μl DMSO and vibrated for 10 min. At last, the plate was read at 570 nm to obtain the absorbance value.

### *TUNEL assay*

The cells were treated by TRAIL at 100 μg/L, 200 μg/L, and 500 μg/L for 12 h, 24 h, and 48 h, respectively. The cells were washed by xylene and gradient ethanol. Next, the cells were treated by Proteinase K and added with 50 μl TUNEL solution. Then the cells were treated by 50 μl converter-POD together with 50-100 μl DAB

**Table 2.** TRAIL affected SGC-7901 cell apoptosis

| Time | Experimental group | | | Control |
|---|---|---|---|---|
| | 100 μg/L | 200 μg/L | 500 μg/L | |
| 12 h | 11.18±1.02[1] | 17.27±1.44[1,2] | 21.24±2.45[1,2,3] | 3.01±1.26 |
| 24 h | 15.21±1.33[1,4] | 18.48±1.67[1,2,4] | 24.37±2.63[1,2,3,4] | 3.21±1.43 |
| 48 h | 18.26±1.54[1,4,5] | 20.85±2.12[1,2,4,5] | 28.92±2.77[1,2,3,4,5] | 3.32±1.39 |

[1]P < 0.05, compared with control; [2]P < 0.05, compared with 100 μg/L; [3]P < 0.05, compared with 200 μg/L; [4]P < 0.05, compared with 12 h; [5]P < 0.05, compared with 24 h.

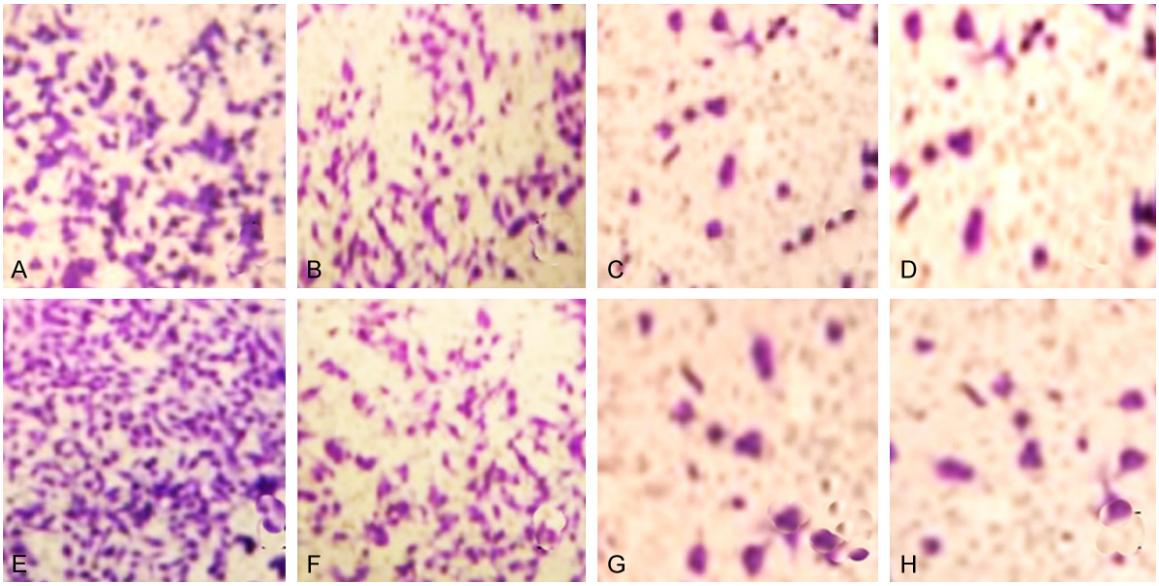


**Figure 2.** TRAIL affected SGC-7901 cell invasion and migration (×400). A. Control; B. 100 μg/L cell invasion; C. 200 μg/L cell invasion; D. 500 μg/L cell invasion; E. Control; F. 100 μg/L cell migration; G. 200 μg/L cell migration; H. 500 μg/L cell migration.

substrate. After redyed by hematoxylin or methyl green, the cells were counted and photographed.

*Transwell assay*

For cell invasion, the matrigel was mixed with serum free medium at 1:8 for 24 h and paved on the chamber. SGC-7901 cells were seeded at $10^6$/ml in 200 μl serum free medium. Another 1300 μl complete medium was added to the lower chamber. After 24 h, the upper chamber was fixed by ethanol and observed under the microscope after staining.

*Western blot*

Total protein was extracted and separated in 8% SDS-PAGE. After blocked at room temperature for 1 h, the membrane was incubated with primary antibodies (Survivin and NF-κB at 1:200, β-actin at 1:500, respectively) at 4°C overnight. After washed by TBS-T, the membrane was incubated in secondary antibody (1:2000) for 1 h. Next, the membrane was treated by developer A and B and scanned. Quantity One software was used for band analysis.

*Data analysis*

SPSS 17.0 software was applied for data analysis. Measurement data were presented as mean ± standard deviation and analyzed by ANOVA. P < 0.05 was depicted as statistical significance.

## Results

*TRAIL affected SGC-7901 cell proliferation*

Different concentrations of TRAIL were sued to treat SGC-7901 cells for 12 h, 24 h, and 48 h. TRAIL significantly inhibited cell proliferation at

**Table 3.** TRAIL affected SGC-7901 cell invasion and migration

| Cell number | Experimental group | | | Control |
|---|---|---|---|---|
| | 100 μg/L | 200 μg/L | 500 μg/L | |
| Invasion | 127.23±15.33[1] | 90.14±4.21[1,2] | 48.12±9.35[1,2,3] | 38.22±2.06 |
| Migration | 181.14±10.83[1] | 99.28±3.61[1,2] | 152.55±7.03[1,2,3] | 37.62±2.86 |

[1]P < 0.05, compared with control; [2]P < 0.05, compared with 100 μg/L; [3]P < 0.05, compared with 200 μg/L.


**Figure 3.** TRAIL affected Survivin and NF-κB protein expressions in SGC-7901 cells.

**Table 4.** TRAIL affected Survivin and NF-κB protein expressions in SGC-7901 cells

| Item | Experimental group | | | Control |
|---|---|---|---|---|
| | 100 μg/L | 200 μg/L | 500 μg/L | |
| Survivin | | | | |
| 12 h | 0.71±0.21[1] | 0.66±0.18[1,2] | 0.16±0.11[1,2,3] | 0.74±0.37 |
| 24 h | 0.54±0.15[1,4] | 0.44±0.13[1,2,4] | 0.10±0.03[1,2,3,4] | 0.72±0.38 |
| 48 h | 0.33±0.13[1,5] | 0.28±0.09[1,2,5] | 0.14±0.01[1,2,3,4,5] | 0.69±0.41 |
| NF-κB | | | | |
| 12 h | 0.53±0.21[1] | 0.47±0.18[1,2] | 0.49±0.11[1,2,3] | 0.10±0.09 |
| 24 h | 0.44±0.25[1,4] | 0.22±0.21[1,2,4,5] | 0.22±0.12[1,2,3,4] | 0.10±0.08 |
| 48 h | 0.26±0.39[1,4,5] | 0.17±0.24[1,2,4,5] | 0.13±0.17[1,2,3,4,5] | 0.11±0.07 |

[1]P < 0.05, compared with control; [2]P < 0.05, compared with 100 μg/L; [3]P < 0.05, compared with 200 μg/L; [4]P < 0.05, compared with 12 h; [5]P < 0.05, compared with 24 h.

different concentrations and times compared with control (P < 0.05). TRAIL intervention at 500 μg/L for 48 h exhibited the strongest suppressive effect on cell proliferation (P < 0.05) (**Table 1**).

*TRAIL affected SGC-7901 cell apoptosis*

TUNEL assay was applied to test the impact of TRAIL on SGC-7901 cell apoptosis. TRAIL obviously promoted cell apoptosis with dose and time dependence (P < 0.05) (**Figure 1** and **Table 2**).

*TRAIL affected SGC-7901 cell invasion and migration*

Transwell assay was adopted to test the influence of TRAIL on SGC-7901 cell invasion and migration. TRAIL markedly suppressed cell invasion and migration with dose and time dependence (P < 0.05) (**Figure 2** and **Table 3**).

*TRAIL affected Survivin and NF-κB protein expressions in SGC-7901 cells*

Western blot was selected to determine Survivin and NF-κB protein expressions in SGC-7901 cells treated by TRAIL. Survivin and NF-κB protein expressions markedly down-regulated in SGC-7901 cells treated by TRAIL with time and dose dependence (P < 0.05) (**Figure 3** and **Table 4**).

## Discussion

Gastric cancer is a common digestive tract malignant tumor. Its occurrence pattern is a multi-stage process. Chronic gastritis may form atrophic gastritis, further leading to intestinal metaplasia and hyperplasia. It occurs canceration eventually, lead to the occurrence of gastric cancer [9]. TRAIL is a member of the tumor necrosis factor family shows high homology with FasL. It can induce most of the tumor cell apoptosis [10, 11]. At present, several scholars proposed that Survivin may be related to the inhibition of apoptosis, the regulation of cell proliferation, and tumor angiogenesis [12]. NF-κB is a type of multifunction transcription factor that plays a central regulatory role in tumor cell proliferation and apoptosis. Its aberrant transcription activation is closely related to the upregulation of anti-apoptotic genes [13]. This study selected gastric cancer cell line SGC-7901 to analyze the impact of TRAIL on Survivin and NF-κB expressions and related mechanism.

Different concentrations of TRAIL were sued to treat SGC-7901 cells for 12 h, 24 h, and 48 h. TRAIL significantly inhibited cell proliferation at different concentrations and times compared

with control. TRAIL intervention at 500 μg/L for 48 h exhibited the strongest suppressive effect on cell proliferation. TUNEL assay was applied to test the impact of TRAIL on SGC-7901 cell apoptosis. TRAIL obviously promoted cell apoptosis with dose and time dependence. Transwell assay was adopted to test the influence of TRAIL on SGC-7901 cell invasion and migration. TRAIL markedly suppressed cell invasion and migration with dose and time dependence. TRAIL significantly inhibited SGC-77901 cell proliferation, facilitated cell apoptosis, and restrained cell invasion and migration. TRAIL belongs to TNF superfamily that can only induce apoptosis in transformed cells, tumor cells, and virus infected cells without normal cells. It was showed that TRAIL has certain apoptosis induction role on different types of gastric cancer cells with time dependence, such as AGS, MKN28, and MKN45 cell lines. However, the response of different cell lines to TRAIL was different, indicating the cell sensitivity to TRAIL was different [14, 15].

To explore the role of TRAIL on Survivin and NF-κB expressions in gastric cancer, this study applied different concentrations of TRAIL to intervene SGC-7901 cells. Survivin and NF-κB protein expressions markedly downregulated in SGC-7901 cells treated by TRAIL with time and dose dependence, suggesting that TRAIL can suppress Survivin and NF-κB expressions in gastric cancer. Survivin was found abnormally upregulated in gastric cancer, and its expression was higher in a tissue approaching the tumor infiltrated region [16]. Wakana collected 21 cases of intestinal type and diffuse type gastric cancer samples and found that Survivin mRNA and protein were negatively correlated with apoptotic index. It exhibited higher positive rate in diffuse type gastric cancer [17]. Overexpression of Survivin can inhibit cell apoptosis, elevate cell malignancy, and promoted cell metastasis in gastric cancer [18]. NF-κB demonstrated increasing trend in normal gastric tissue, adjacent tissue, and cancer tissue. Its excessive activation plays a role in the early and progressive stages of cancer [19, 20]. NF-κB suppresses gastric cancer apoptosis and facilitates cell proliferation and metastasis through regulating the downstream anti-apoptotic genes [21-23]. Our results suggested that TRAIL significantly inhibited SGC-77901 cell proliferation, facilitated cell apoptosis, and restrained cell invasion and migration through suppressing Survivin and NF-κB.

TRAIL significantly inhibited SGC-77901 cell proliferation, facilitated cell apoptosis, and restrained cell invasion and migration through suppressing Survivin and NF-κB. It is worth of promoting Survivin and NF-κB detection in gastric cancer patients, especially in those received surgery, chemotherapy, or radiotherapy. Monitoring their expressions before and after treatment, together with clinicopathological characteristics may provide appropriate evaluation for the prognosis. More in-depth investigation on the aspects of animal model, gene, and protein may supply better therapeutic choice to improve the prognosis. The process of gastric cancer occurrence involves multiple cytokines with complex interactions. Precise detection of Survivin and NF-κB expressions may provide new strategy for the treatment of gastric cancer. Its specific mechanism still needs further exploration.

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## Disclosure of conflict of interest

None.

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## References

[1] Tsai HF, Hsu PN. Modulation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis by helicobacter pylori in immune pathogenesis of gastric mucosal damage. J Microbiol Immunol Infect 2017; 50: 4-9.

[2] Lim SC, Han SI. Ursodeoxycholic acid effectively kills drug-resistant gastric cancer cells through induction of autophagic death. Oncol Rep 2015; 34: 1261-1268.

[3] Zhu X, Zhang K, Wang Q, Chen S, Gou Y, Cui Y, Li Q. Cisplatin-mediated c-myc overexpression and cytochrome c (cyt c) release result in the up-regulation of the death receptors DR4 and DR5 and the activation of caspase 3 and cas-

pase 9, likely responsible for the TRAIL-sensitizing effect of cisplatin. Med Oncol 2015; 32: 133.
[4] Cui YF, Yu LS, Wang HQ, Gou YW, Wang QM, Zhang KG. Effect of TRAIL in combination with DDP on the expression of MDR1 gene in gastric cancer cells. Prz Gastroenterol 2014; 9: 214-219.
[5] Zhu H, Huang M, Ren D, He J, Zhao F, Yi C, Huang Y. The synergistic effects of low dose fluorouracil and TRAIL on TRAIL-resistant human gastric adenocarcinoma AGS cells. Biomed Res Int 2013; 2013: 293874.
[6] Ahmed F, Toume K, Ohtsuki T, Rahman M, Sadhu SK, Ishibashi M. Cryptolepine, isolated from Sida acuta, sensitizes human gastric adenocarcinoma cells to TRAIL-induced apoptosis. Phytother Res 2011; 25: 147-150.
[7] Suganya G, Bavle RM, Paremala K, Makarla S, Sudhakar M, Reshma V. Survivin expression in oral lichen planus: role in malignant transformation. J Oral Maxillofac Pathol 2016; 20: 234-238.
[8] Boonyarat C, Yenjai C, Vajragupta O, Waiwut P. Heptaphylline induces apoptosis in human colon adenocarcinoma cells through bid and Akt/NF-kappaB (p65) pathways. Asian Pac J Cancer Prev 2014; 15: 10483-10487.
[9] Fuentes RG, Toume K, Arai MA, Sadhu SK, Ahmed F, Ishibashi M. Scopadulciol, isolated from scoparia dulcis, induces beta-catenin degradation and overcomes tumor necrosis factor-related apoptosis ligand resistance in AGS human gastric adenocarcinoma cells. J Nat Prod 2015; 78: 864-872.
[10] Wang W, Zhao J, Wang H, Sun Y, Peng Z, Zhou G, Fan L, Wang X, Yang S, Wang R, Fang D. Programmed cell death 4 (PDCD4) mediates the sensitivity of gastric cancer cells to TRAIL-induced apoptosis by down-regulation of FLIP expression. Exp Cell Res 2010; 316: 2456-2464.
[11] Kimberley FC, Screaton GR. Following a TRAIL: update on a ligand and its five receptors. Cell Res 2004; 14: 359-372.
[12] Li Y, Zhou Y, Zheng J, Niu C, Liu B, Wang M, Fang H, Hou C. Downregulation of survivin inhibits proliferation and migration of human gastric carcinoma cells. Int J Clin Exp Pathol 2015; 8: 1731-1736.
[13] Lin CL, Chen RF, Chen JY, Chu YC, Wang HM, Chou HL, Chang WC, Fong Y, Chang WT, Wu CY, Chiu CC. Protective effect of caffeic acid on paclitaxel induced anti-proliferation and apoptosis of lung cancer cells involves NF-kappaB pathway. Int J Mol Sci 2012; 13: 6236-6245.
[14] Abdelfattah MS, Toume K, Ishibashi M. Yoropyrazone, a new naphthopyridazone alkaloid isolated from streptomyces sp. IFM 11307 and evaluation of its TRAIL resistance-overcoming activity. J Antibiot (Tokyo) 2012; 65: 245-248.
[15] Toume K, Tsukahara K, Ito H, Arai MA, Ishibashi M. Chromomycins A2 and A3 from marine actinomycetes with TRAIL resistance-overcoming and Wnt signal inhibitory activities. Mar Drugs 2014; 12: 3466-3476.
[16] Tong WW, Tong GH, Chen XX, Zheng HC, Wang YZ. HIF2alpha is associated with poor prognosis and affects the expression levels of survivin and cyclin D1 in gastric carcinoma. Int J Oncol 2015; 46: 233-242.
[17] Hino R, Uozaki H, Inoue Y, Shintani Y, Ushiku T, Sakatani T, Takada K, Fukayama M. Survival advantage of EBV-associated gastric carcinoma: survivin up-regulation by viral latent membrane protein 2A. Cancer Res 2008; 68: 1427-1435.
[18] Kinoshita J, Fushida S, Harada S, Yagi Y, Fujita H, Kinami S, Ninomiya I, Fujimura T, Kayahara M, Yashiro M, Hirakawa K, Ohta T. Local angiotensin II-generation in human gastric cancer: correlation with tumor progression through the activation of ERK1/2, NF-kappaB and survivin. Int J Oncol 2009; 34: 1573-1582.
[19] Sun Y, Zhao Y, Hou L, Zhang X, Zhang Z, Wu K. RRR-alpha-tocopheryl succinate induces apoptosis in human gastric cancer cells via the NF-kappaB signaling pathway. Oncol Rep 2014; 32: 1243-1248.
[20] Liu AQ, Xie ZQ, Chen XN, Feng J, Chen JW, Qin FJ, Ge LY. Fas-associated factor 1 inhibits tumor growth by suppressing helicobacter pylori-induced activation of NF-kappaB signaling in human gastric carcinoma. Oncotarget 2016.
[21] He G, Li LI, Guan E, Chen J, Qin YI, Xie Y. Fentanyl inhibits the progression of human gastric carcinoma MGC-803 cells by modulating NF-kappaB-dependent gene expression in vivo. Oncol Lett 2016; 12: 563-571.
[22] Han MA, Lee DH, Woo SM, Seo BR, Min KJ, Kim S, Park JW, Kim SH, Choi YH, Kwon TK. Galangin sensitizes TRAIL-induced apoptosis through down-regulation of anti-apoptotic proteins in renal carcinoma Caki cells. Sci Rep 2016; 6: 18642.
[23] Kurbanov BM, Fecker LF, Geilen CC, Sterry W, Eberle J. Resistance of melanoma cells to TRAIL does not result from upregulation of antiapoptotic proteins by NF-kappaB but is related to downregulation of initiator caspases and DR4. Oncogene 2007; 26: 3364-3377.