Changes of NF-κB, p53, Bcl-2 and caspase in apoptosis induced by JTE-522 in human gastric adenocarcinoma cell line AGS cells: role of reactive oxygen species

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Abstract
AIM: To identify whether JTE-522 can induce apoptosis in AGS cells and ROS also involved in the process, and to investigate the changes in NF-κB, p53, bcl-2 and caspase in the apoptosis process.

METHODS: Cell culture, MTT, Electromicroscopy, agarose gel electrophoresis, lucigenin, Western blot and electrophoretic mobility shift assay (EMSA) analysis were employed to investigate the effect of JTE-522 on cell proliferation and apoptosis in AGS cells and related molecular mechanisms.

RESULTS: JTE-522 inhibited the growth of AGS cells and induced the apoptosis. Lucigenin assay showed the generation of ROS in cells under incubation with JTE-522. The increased ROS generation might contribute to the induction of AGS cells to apoptosis. EMSA and Western blot revealed that the level of bcl-2 was decreased, whereas p53 showed a great increase following JTE-522 treatment. Their changes were in a dose-dependent manner.

CONCLUSION: These findings suggest that reactive oxygen species, NF-κB, p53, bcl-2 and caspase-3 may play an important role in the induction of apoptosis in AGS cells after treatment with JTE-522.

INTRODUCTION
Apoptosis is an active cell death process, which requires specific gene regulation. A critical role for p53 in the execution of some forms of apoptosis has been suggested. This protein is a sequence-specific DNA-binding protein, active as a transcription factor. It has been proposed that p53 may be involved in the cellular response to DNA damage, producing arrest in the G1 phase of the cell cycle to allow efficient repair of DNA before entry to S phase, or cell death if the damage is too large to be repaired. Another gene implicated in apoptosis is bcl-2. The bcl-2 gene product functions as an anti-apoptotic signal, suppressing apoptosis induced by a wide variety of stimuli, including chemotherapeutic drugs and γ radiation. The exact mechanism of bcl-2 in preventing apoptosis is still not clear. However, bcl-2 has been implicated in cellular control of their redox state.

Previous studies have demonstrated that non-steroidal anti-inflammatory drugs (NSAIDs) given in vivo to rodents and human can inhibit tumor growth. JTE-522 is a novel NSAID, which is a specific inhibitor of cyclooxygenase-2 (COX-2) with significant anti-inflammatory and analgesic properties. Some reported that JTE-522 possesses strong chemopreventive activity against colon carcinogenesis, but the precise mechanism by which JTE-522 inhibits colon carcinogenesis is not clear. It is often attributed to specific inhibition of arachidonic acid metabolism via coxenzymes. However, recent studies showed that the antitumor effect had little connection with NSAIDs inhibitory activity against cyclooxygenase, and was not prevented by exogenous supplementation of 16,16-dimethyl prostaglandin E2. Several groups have shown that certain NSAIDs induce apoptosis of tumor cell line, which is associated with the generation of reactive oxygen species (ROS). However, the signaling pathway leading to apoptotic cell death remains unclear.

ROS can play a central role in regulating cell proliferation and cell death. Evidence has been obtained that ROS such as superoxide and hydrogen peroxide can influence cell death triggered by internal cues (p53-mediated), external cues (TGF-beta-mediated) and immunogenic signals (TNF-alpha). In other instances, however, generation of ROS can inhibit apoptosis. Although the mechanism involved is still controversial, redox status and/or hydrogen peroxide have both been proposed as critical factors. Therefore it is possible that ROS may play a role in regulating apoptosis in gastric epithelium.

The purpose of the present study is to identify whether JTE-522 can induce apoptosis in AGS cells and ROS are also involved in the process, and to investigate the changes in NF-κB, p53, bcl-2 and caspase in the apoptosis process.

MATERIALS AND METHODS
Cell line and reagents
Human gastric adenocarcinoma cell line AGS was provided by Cancer Institute, Zhongshan University. Cells were grown in RPMI-1640 medium and supplemented with 10% new bovine serum, penicillin G (100kU.L") and kanamycin (0.1 g.L") at 37°C in a 5% CO2-95% air atmosphere. Antibodies used in this study included p53, bcl-2, IkBα and Beta actin were obtained from Santa Cruz. All other chemicals were purchased from Sigma Chemical Co (St. Louis, MO, USA).

MTT assay
AGS cells growing on 96-well plates were treated with JTE-522 (0.1 mmol/L - 1 mmol/L) for 72h, untreated cells served as a control. 10µL of the 2.5g.L-1 stock solution of 3-[4, 5-dimethylthiaoyl]-2, 3-diphenyl tetrazolium bromide (MTT) was added to each well and was incubated for 4h at 37°C. 100 µL of DMSO was added to dissolve the formazan crystals formed. The absorbance at 570nm was measured on a microplate reader. The absorbance of the test group was divided by the control group as the relative optical density (RD).
5-diphenyl-tetrazolium bromide (MTT) was added to each well. After 1h of incubation at 37°C, the medium was removed. 50µl of the extraction buffer (10% Triton-X100, 0.1mol/L HCl) was added, and plates were gently shaken for 30min at room temperature. The optical densities were measured at 570nm.

Morphological and biochemical analysis of apoptosis
Morphological changes in the nuclear chromatin of cells undergoing apoptosis were detected by electron microscopy (EM). Cells were pelleted and fixed with 30mL/L glutaraldehyde in PBS. EM analysis was performed as described previously[22]. Oligonucleosomal cleavage of genomic DNA was detected by agarose gel electrophoresis. In brief, genomic DNA isolated as previously described[24] was subjected to 1.5% agarose gel electrophoresis, followed by ethidium bromide staining.

Assay for reactive oxygen species production
Generation of ROS was assessed using lucigenin. AGS cells grown in 75cm² culture flasks were incubated for 6h with JTE-522 (0.1-1mmol/L) in the presence or absence of 100µmol/L pyrrolidine dithiocarbamate (PDTC). The cells were then scraped off and washed in cold Hank’s buffer. An aliquot containing 1×10⁶ cells in 100µL of Hank’s buffer was mixed in microtitrator wells with 100µl of lucigenin prepared at a concentration of 40µmol/L. Light emission was detected using a Berthold LB96V luminometer for 3min.

Assessment of caspase activity
Caspase-3 activity was measured using a caspase assay kit according to the supplier’s instruction. In brief, caspase-3 fluorogenic substrates (Ac-DEVD-AMC or Ac-IETD-AMC) were incubated with JTE-522-treated with cell lysates for 1h at 37°C, then AMC liberated from Ac-DEVD-AMC or Ac-IETD-AMC was measured using a fluorometric plate reader with an excitation wavelength of 380nm and an emission wavelength of 420-460nm.

Western blot analysis
The cells were lysed in lysis buffer (25mmol/L hepes, 1.5% Triton X-100, 1% sodium deoxycholate,0.1% SDS, 0.5mol/L NaCl, 5mmol/L EDTA, 50mmol/L NaF, 0.1mmol/L sodium vanadate,1mmol/L phenylmethylsulfonyl fluoride(PMSF),and 0.1g/L 1 leupeptin (pH7.8) at 4°C with sonication. The lysates were centrifuged at 15000g for 15min and the concentration of the protein in each lysate was determined with Coomassie brilliant blue G-250. Loading buffer (42mmol/L Tris-HCl, 10% glycerol, 2.3% SDS, 5% 2-mercaptoethanol and 0.002% bromophenol blue) was then added to each lysate, which was subsequently boiled for 3min and then electrophoresed on a SDS-polyacrylamide gel. Proteins were transferred to nitrocellulose and incubated sequentially with antibodies against IkBα, p53 and bcl-2 and then with peroxidase-conjugated secondary antibodies in the second reaction. Detection was performed with enhanced chemiluminescence reagent.

Electrophoretic mobility shift assay (EMSA)
Nuclear extracts were prepared from AGS cells treated with JTE-522. Synthetic double-strand oligonucleotides of consensus NF-kB binding sequence, GATCCCAACGGCAGGGGA, were end-labeled with [γ³²P]ATP using T4 polynucleotide kinase. Nuclear extract was incubated with the labeled probe in the presence of poly (dl-dC) in a binding buffer containing 20mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid at room temperature for 30min. For supershift assays, a total of 0.2µg of antibodies against p65 subunit of NF-kB were included in the reaction. DNA-protein complexes were resolved by electrophoresis in a 5% non-denaturing polyacrylamide gel, which was dried and visualized by autoradiography.

RESULTS

Effect of JTE-522 on cell proliferation and apoptosis
AGS cells were incubated with various does of JTE-522 for 72h. Analysis of cell viability using MTT assay showed that JTE-522 significantly inhibited cell viability. The inhibition of cell viability was dependent on the dose of JTE-522 used (Figure 1).

![Figure 1](image1.png)  Effect of JTE-522 on cell growth in AGS cells. The cells were treated with various concentrations of JTE-522 for 72h. The antiproliferative effect was measured by MTT assay. Results are the means±SD from three independent determinations.

The effect was due to apoptosis as demonstrated by EM and electrophoresis of genomic DNA. JTE-522-treated cells showed compacted nuclear chromatin with finely granular masses marginated against the nuclear envelope and condensed cytoplasm, the nuclear outline was convoluted and the organelles were preserved (Figure 2) and led to oligonucleosomal cleavage of genomic DNA (Figure 3), which were hallmarks of apoptosis.

![Figure 2](image2.png)  Electro micrographs of JTE-522-treated AGS cells. Control AGS cells (A), or treated with 1mmol/L JTE-522 for 72h, were examined by EM as in “Materials and Methods”. Magnification: ×4000
We next investigated whether the activation of caspase was involved in JTE-522-induced apoptosis of AGS cells. JTE-522-induced apoptosis of AGS cells was accompanied by the induction of caspase activity as demonstrated by the cleavage of Ac-DEVD-AMC and Ac-IETD-AMC, respectively (Figure 4). These results indicated that JTE-522-induced cell death of AGS cells was a typical apoptosis associated with caspase activation.

![Figure 4](https://example.com/figure4.png)

**Figure 4** Activation of caspase-3 activities by JTE-522 in AGS cells for indicated time period. JTE-522 treatment (0.75mmol/L) induced cleavage of Ac-DEVD-AMC and Ac-IETD-AMC, indicating activation of caspase-3 activity, respectively.

**Effect of JTE-522 on the production of ROS in AGS cells**

The effect of JTE-522 on the production of ROS in AGS cells, as assessed with lucigenin chemiluminescence, was shown in Figure 5. Chemiluminescence was significantly enhanced by incubation with various doses of JTE-522. This enhancement was prevented by co-incubation with PDTC at 100µmol/L. These results demonstrated the generation of reactive oxygen species in cells under incubation with JTE-522.

![Figure 5](https://example.com/figure5.png)

**Figure 5** Effect of JTE-522 on the generation of ROS. AGS cells were incubated for 6h with JTE-522 (0.25-1mmol/L) in the presence or absence of PDTC at 100µmol/L. Lucigenic-associated chemiluminescence was measured for 3min with a lumimeter. (A). Lane 1: control; lane 2-5: AGS cells treated with 0.25, 0.5, 0.75, 1mmol/L of JTE-522; lane 6: JTE-522 (1mmol/L) +PDTC (100µmol/L). *P*<0.01 vs control. Results are the means±SD from three independent determinations.

**Effect of JTE-522 on the expression of p53, bcl-2, IkBα and the activation of NF-kB**

NF-kB plays a complex role in regulating programmed cell death. In many instances the inhibition of NF-kB activity can sensitize cells to death inducers[25]. In other instances, however, NF-kB activation has been found to play an important role in the induction of apoptosis. To determine whether the treatment with JTE-522 have any effect in the NF-kB transcriptional factors. We performed EMSA with nuclear extracts prepared from control or treated cells exposed to JTE-522 for various concentrations for 6h. The NF-kB specific complexes found in this cell line were almost complete inhibited in comparision with untreated cells (Figure 6A). In accordance with result, an analysis of IkBα proteins level by Western blot demonstrated that the degradation of this protein was greatly inhibited during the apoptotic process(Figure 6B).

Additionally, by using Western blot we confirmed that the level of bcl-2 was decreased, whereas p53 showed a great increase following JTE-522 treatment. Their changes were in a dose-dependent manner (Figure 7).

![Figure 6](https://example.com/figure6.png)

**Figure 6** Effect of JTE-522 on NF-kB binding activity and IkBα degradation. Cells were treated with JTE-522 for 6h. Cells were harvested and EMSA was performed as described (A). Lane 1: control; lane 2-5: AGS cells treated with 0.25, 0.50, 0.75, 1mmol/L. of JTE-522. The identity of DNA-complexed proteins was confirmed by supershift assays using antibodies against p65 subunit of NF-kB (lane 6). Immunoblot analysis of IkBα of corresponding cytosolic supernatant (B). Representative results from four independent experiments.

![Figure 7](https://example.com/figure7.png)

**Figure 7** P53, bcl-2 protein levels in AGS cells treated with JTE-522. Cell lysates were collected and processed at 6h. The whole cellular protein was electrophoresed in SDS-PAGE gel. Western blot was performed using antibodies against p53, bcl-2. Beta actin was used as a lane-loading control. (1) control; (2) 0.25mmol/L; (3) 0.5mmol/L; (4) 0.75mmol/L; (5) 1mmol/L. Representative results from three independent experiments.
DISCUSSION

Using cultured AGS human gastric adenocarcinoma cells, the observations described in this study demonstrate that JTE-522, a novel NSAIDs inhibits the growth of AGS cells and induces apoptosis in a concentration-dependent manner.

The onset of apoptosis is associated with the proteolytic activation of caspases. Caspases, a family of cysteine proteases, play a critical role in the execution of apoptosis[20-22]. They are synthesized as proenzymes that are processed by self-proteolysis and/or cleavage by another protease to their active forms in cells undergoing apoptosis. Caspase-3 is a major executioner of apoptosis. It is promoted during the early stage of apoptosis and the activated form is a marker for cells undergoing apoptosis[23]. After activation by initiators, the proform (p32) is cleaved to the active forms p20, p17, or p11, respectively[24-25]. Therefore, activation of caspase-3 in AGS cells in this study not only indicated the occurrence of apoptosis, but also implied the involvement of caspase-3 in JTE-522-induced apoptosis.

ROS have been found to play a central role in regulating apoptosis in numerous instances. Given that reduced rates of apoptosis may contribute to carcinogenesis, the regulation of cellular ROS production may be an important variable in the development of neoplasias. Other studies have also suggested a potential role for ROS in cancer suppression. For example, the p53 tumor suppressor protein activates the expression of ROS-generating proteins that increase cellular ROS production and eventually trigger apoptosis[26-28]. Increased ROS generation by chemopreventive agents may serve to compensate the lower levels of ROS generation in p53 null cells (AGS cells are p53 null)[29-30]. Animal studies have also implicated ROS in regulating carcinogenesis. Mice with elevated levels of glutathione peroxidase are more sensitive to skin carcinogenesis than their wild type counterpart[31]. A similar correlation has been made in the colon, where strains with higher levels of glutathione peroxidase activity have a higher cancer risk. The role of ROS in carcinogenesis is however likely to be complex given the potential mutagenic activity of ROS. For example, the NSAIDs inhibition of cyclooxygenase has been proposed to suppress carcinogenesis by suppressing the production of peroxyl radicals and the subsequent formation of mutagenic lipid peroxidation breakdown products[32]. The role of ROS in carcinogenesis may depend on the relative levels of different ROS generated, and where and when they are present. However, as demonstrated in this paper, we showed that JTE-522 increased ROS generation in AGS cells, and this increased ROS generation might contribute to the induction of AGS cells to apoptosis.

The bcl-2 protooncogene is unique among cellular genes for its ability in many contexts to block apoptotic cell death. A mechanism has been proposed in which bcl-2 regulates antioxidant pathways at sites of free radical generation[33]. The protein of bcl-2 also protects against apoptosis by blocking cytochrome C release hence this protein may have an antioxidant function[34]. In our experiment, the expression of bcl-2 was reduced in AGS cells treated with JTE-522. Western blot experiments demonstrated that this effect was mediated by inhibition of IκBα degradation. Determining the role of NF-κB in gastric carcinogenesis could help to guide the development of improved chemoprevention and treatment strategies.

In summary, JTE-522 inhibited cell growth and induced apoptosis in AGS cells. Increased ROS may play an important role in this caspase-3 mediated apoptotic process. The inhibition of NF-κB by JTE-522 may be mediated by preventing IκBα degradation. The precise relationship and importance of each of these factors in the apoptotic process should be established by more direct and profound analysis.

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REFERENCES

1 Peng XM, Peng MM, Chen Q, Yao JL. Apoptosis, Bcl-2 and p53 protein expression in tissues from hepatocellular carcinoma. Huaren Xixia Zazhi 1998; 6: 834-836
2 Hua JS. Effect of Hp: cell proliferation and apoptosis on stomach cancer. ShiJie Huaren Xixia Zazhi 1999;7: 647-648
3 Xue XC, Fang GE, Hua JD. Gastric cancer and apoptosis. ShiJie Huaren Xixia Zazhi 1999;7: 359-361
4 Qin LF, Wang RN. Prognostic significance of FCM DNA analysis in carcinoma of stomach. Shanghai Dier Yi Ke Daxue Xuebao 1992; 12: 196-202
5 Yu GQ, Zhou Q, Ding Ivan, Gao SS, Zhang ZY, Zou JX, Li YX, Wang LD. Changes of p53 protein blood level in esophageal cancer patients and normal subjects from a high incidence area in Henan, China. World J Gastroenterol 1998: 4: 218
6 Luo D, Liu QF, Gove V, Namon NV, Su JF, Williams R. Analysis of N-ras gene mutation and p53 gene expression in human hepatocellular carcinomas. World J Gastroenterol 1998; 4: 97-99
7 Li H, Zhang HW, Ren XD. Synergism between heparin and adriamycin on cell proliferation and apoptosis in human nasopharyngeal carcinoma CNE2 cells. Aca Pharmacol sin 2002;23:167-172
8 Li HL, Ye KH, Ren XD. Heparin induced apoptosis in human nasopharyngeal carcinoma CNE2 cells. Cell Research 2001;11:311-315
9 Qiao Q, Wu JS; Zhang J, Ma QJ, Lai DN. Expression and significance of apoptosis related gene bcl-2, bax in human large intestine adenocarcinoma. ShiJie Huaren Xixia Zazhi 1999; 7: 936-938
10 Yuan RW, Ding Q, Jiang HY, Qin X, Zou SQ, Xia SS. Bcl-2, p53 protein expression and apoptosis in pancreatic cancer. ShiJie Huaren Xixia Zazhi 1999; 7: 851-854
11 Jiang YG, Li QF, Wang YG, Gu CH. Bcl-2/bax expression and hepatocyte apoptosis on liver tissue in tupaia with HDV/HBV infection. ShiJie Huaren Xixia Zazhi 2000; 8: 625-628
12 Fan NQ, Ya JG. Apoptosis in oncology. Cell Research 2001;11:1-7
13 Zhang MH, Zhang Q, Shao BX. Effect of Bcl-2 and caspase-3 on calcium distribution in apoptosis of HL-60 cell. Cell Research 2000; 10: 213-20
14 Kane DJ, Sarafian TA, Anton R, Halh H, Buttler E. Bcl-2, inhibition of neural death: decreased generation of reactive oxygen species. Science 1993; 262: 1274-1278
15 Reddy BS, Rao CV, Seibert K. Evaluation of COX-2 inhibitor for potential chemopreventive properties in colon carcinogenesis. Cancer Res 1996; 56: 4566-4569
16 Shibata MA, Hasegawa R, Imaida K, Hagiwara A. Chemoprevention by dehydroepiandrosterone and indomethacin in a rat model of multigorgan carcinogenesis model. Cancer Res 1995; 55: 4870-4874
17 Tomozawa S, Nagawa H, Tsuno N. Inhibition of haematogenous metastasis of colon cancer in mice by a selective COX-2 inhibitor, JTE-522. Br J Cancer 1999; 81: 1274-1279
18 Yang ZY, Borton KA. Cyclooxygenase-2-selective antagonists do not inhibit growth of colorectal carcinoma cell lines. Cancer letters 1998; 122: 25-30
Kusuhara H, Komatsu H, Sugahara K. Reactive oxygen species are involved in the apoptosis induced by NSAIDs in cultured gastric cells. *Eur J Pharmacol* 1999; 383: 331-337

Tanaka K, Pracyk JB, Takeda K, Yu ZX, Finkel T. Expression of Id1 results in apoptosis of cardiac myocytes through a redox-dependent mechanism. *J Biol Chem* 1998; 273: 25922-25928

Fridovich I. Superoxide radical and superoxide dismutases. *Annu Rev Biochem* 1995; 64: 97-112

Manna SK, Zhang HJ, Yan T, Oberley LW, Aggarwal BB. Overexpression of manganese superoxide dismutase suppress tumor necrosis factor-induced apoptosis and activation of nuclear transcription factor-b and AP-1. *J Biol Chem* 1998; 273: 13245-13254

Qiao L, Hanif R, Spichal E, Steven J, Rigas B. Effect of aspirin on induction of apoptosis in AGS human colon adenocarcinoma cells. *Biochem Pharmacol* 1998; 55: 53-64

Jiang ZF, Zhao Y, Hong X, Zhai ZH. Nuclear apoptosis induced by isolated mitochondria. *Cell Research* 2000; 10: 221-232

Beg AA, Baltimore D. An essential role for NF-kB in preventing TNF-alpha induced cell death. *Science* 1996; 274: 787-789

Cohen GM. Caspases: the executioners of apoptosis. *Biochem J* 1997; 326: 1-6

Kumar S, Lavin MF. The ICE family of cysteine proteases as effectors of cell death. *Cell Death Differ* 1996; 3: 255-267

Salvesen GS and Dixit VM. Caspases: intracellular signaling by proteolysis. *Cell* 1997; 91: 443-446

Shen ZY, Shen J, Li QS, Chen CY, Chen JY, Zeng Y. Morphological and functional changes of mitochondria in apoptosis esophaged carcinoma cells induced by arsenic trioxide. *World J Gastroenterol* 2002; 8: 31-35

Du C, Fang M, Li Y, Wang X. Smac A. Mitochondrial protein that promotes cytochrome c dependent caspase activation by eliminating IAP inhibition. *Cell* 2000; 102: 43-53

Desagher S, OsenSand A, Nichols A, Eskes R, Montessuit S, Lauper S, Maundrell K, Antonsson B, Martinou JC. Bid-induced conformational cytochrome c release during apoptosis. *J Cell Biol* 1999; 144: 891-903

Schlegel J, Peters I, Orrenius S, Miller DK. Cyp32/apopain is a key interleukin 1 beta converting enzyme-like protease involved in Fas-mediated apoptosis. *J Biol Chem* 1996; 271: 1841-1844

Stennicke HR, Salvesen GS. Properties of the caspases. *Biochim Biophys Acta* 1998; 1437: 17-31

Polyak K, Xia Y, Zweier JL, Kinzler KW. A model for p53-mediated apoptosis. *Nature* 1997; 389: 300-305

Johnson TM, Yu ZX, Ferrans RA. Relative oxygen species are downstream mediators of p53-dependent apoptosis. *Proc Natl Acad Sci USA* 1996; 93: 11848-11852

Ossina NK, Cannas A, Powers VC, Gilbert EM, Tomei SR. Interferon-gamma modulates a p53-independent apoptotic pathway and apoptosis-related gene expression. *J Biol Chem* 1997; 272: 16351-16357

Xu CT, Huang LT, Pan BK. Current gene therapy for stomach carcinoma. *World J Gastroenterol* 2001; 7: 752-759

Lu YP, Lou YR, Newmark HL, Huang MF. Enhanced skin carcinogenesis in transgenic mice with high expression of glutathione peroxidase or both glutathione peroxidase and superoxide dismutase. *Cancer Res* 1997; 57: 1468-1474

Hockenbery OM, Oltvai ZN, Yin XM, Korsmeyer SJ. Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* 1993; 75: 241-251

Cai J, Jones DP. Superoxide in apoptosis: mitochondrial generation triggered by cytochrome C loss. *J Biol Chem* 1998; 273: 11401-1144

Barnes PJ, Karin M. Nuclear factor-kB, a pivotal transcription factor in chronic inflammatory disease. *New Eng J Med* 1997; 336: 1066-1071

Huang S, Li JY, Wu J, Meng L, Shou CC. Mycoplasma infections and different human carcinomas. *World J Gastroenterol* 2001; 7: 266-269

Baueuerle PA, Baltimore D. NF-kB: ten years after. *Cell* 1996; 87: 13-20

Yu YL, Sun B, Zhang XJ, Wang SN, He HY, Qiao MM, Zhong J, Xu JY. Growth inhibition and apoptosis induction of Sulindac on human gastric cancer cells. *World J Gastroenterol* 2001; 7: 796-800

Kipp E, Ghosh S. Inhibition of NF-kB by sodium salicylate and aspirin. *Science* 1994; 265: 956-959

Giardina C, Boudares H, Ihan MS. NSAIDs and butyrate sensitize a human colorectal cancer cell line to TNF and Fas ligation: the role of reactive oxygen species. *Biochim Biophys Acta* 1999; 1448: 425-438

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