Tanshinone IIA Inhibits Gastric Carcinoma AGS Cells Through Increasing p-p38, p-JNK and p53 but Reducing p-ERK, CDC2 and Cyclin B1 Expression

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Abstract. *Tanshinone IIA (Tan-IIA) is extracted from Danshen (Salviae miltiorrhizae radix). It possesses antitumor activity against a variety of human cancer cells and its induction of apoptosis and inhibition of proliferation of gastric cancer cells are well-documented. However, the molecular mechanisms by which Tan-IIA inhibits gastric cancer have not been well-elucidated. In the present study, we evaluated the cytotoxicity of Tan-IIA against human gastric cancer AGS cells by the (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) MTT assay. The protein expression of tumor necrosis factor-alpha (TNF-α), FAS, p53, p21, cyclin A, cyclin B1, extracellular-related kinase (ERK), phospho extracellular-related kinase (p-ERK), p38, p-p38, Jun-amino-terminal kinase (JNK), phospho Jun-amino-terminal kinase (p-JNK) and β-actin in AGS cells were measured by western blotting. The cell-cycle distribution was analyzed by flow cytometry. The results showed that Tan-IIA inhibited AGS cells with time- and dose-dependent manners. AGS cells treated with Tan-IIA up-regulated the protein expression of TNFα, FAS, p-p38, p-JNK, p53, p21, caspase-3 and caspase-8 but reduced that of p-ERK, CDC2, cyclin A, and cyclin B1. The results also showed that Tan-IIA dose dependently induced G_2/M phase arrest. These findings demonstrate that Tan-IIA can inhibit AGS human gastric cancer cells; one of the molecular mechanisms may be through increasing the protein expression of p-p38 and p-JNK, but decreasing that of p-ERK to induce the activation of p53, followed by increasing the protein expression of p21 to down-regulate CDC2 and cyclin B1 expression which then induces G_2/M phase arrest. Another route may be through increasing the protein expression of TNF-α, FAS, caspase-8 and caspase-3 to induce apoptosis.*

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Key Words: Tanshinone IIA, gastric carcinoma AGS cells, MAPK, G_2/M arrest.

Tanshinone IIA (Tan-IIA; $C_{19}H_{18}O_3$), is extracted from Danshen (Salviae miltiorrhizae radix), and possesses an antitumor activity against many human cancer cells (1, 2), such as colon (3), breast (4) and lung (5) cancer. Tan-IIA induced G_0/G_1 arrest and exerted powerful inhibitory effects against SGC7901 gastric cancer cells time- and dose-dependently (6). Tan-IIA can also reverse the malignant phenotype of SGC7901 gastric cancer cells and induce their apoptosis (7). Chen *et al*. documented that Tan-IIA induced growth inhibition and apoptosis of gastric cancer *in vitro* and *in vivo* (8). It is well-documented that Tan-IIA can up-regulate p53 and down-regulate B-cell leukemia/lymphoma 2 (Bcl-2) gene expression, and arrest MKN-45 gastric cancer cells in the G_2/M phase (9). Our previous study also showed that Tan-IIA can inhibit AGS human gastric cancer cells through decreasing the protein expression of binding immunoglobulin protein (BIP) to induce the activation of endoplasmic reticulum (ER) stress and by decreasing the protein expression of myeloid cell leukemia 1 (Mcl-1), B-cell lymphoma-extra-large (BCL-xL) and translationally controlled tumor protein (TCTP), but increasing that of BCL2-associated X protein (BAX), caspase-9 and caspase-3 (unpublished data).These documents suggest that Tan-IIA may be have potential as a complementary medicines for gastric cancer. FAS (a death domain containing receptor) and tumor necrosis factor-alpha (TNFα) can activate the extrinsic apoptotic pathway (10, 11).The RAS/RAF/mitogen-activated protein kinases (RAS/RAF/MAPK) pathway regulates a variety of cellular functions that are important for tumorigenesis. MAPK, also called MEK, is a serine/threonine kinase activated in response to multiple signals to promote cell survival and apoptosis (12). The

molecular mechanisms of Tan-IIA action against gastric cancer cells remains unclear. In the present study, we investigated TNFα-, FAS- and MAPK-related protein expression in AGS human gastric cancer cells treated with Tan-IIA.

Materials and Methods

The AGS human gastric adenocarcinoma cell line (BCRC number: 60102) was obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan, ROC); Tan-IIA was obtained from Sigma-Aldrich (CAS-No 568-72-9; St. Louis, MO, USA); [3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide] (MTT), sodium deoxycholate, leupeptin, Triton X-100, Tris-HCl, ribonuclease-A, sodium pyruvate, HEPES, dimethyl sulfoxide (DMSO) and Tween-20, and mouse anti-β-actin were obtained from Sigma-Aldrich (St. Louis, MO, USA). Potassium phosphate and 0.2 mm polyvinylidene difluoride (PVDF) membranes were purchased from Merck Co. (Darmstadt, Germany). F-12K medium, fetal bovine serum (FBS), penicillin-streptomycin and glutamine were obtained from Gibco BRL (Grand Island, NY, USA). BioMax film was obtained from Kodak.

Primary antibody. Antibodies against TNFα (cell signaling #3707, MW 25kDa), Fas (Cell signaling #8023, MW 40kDa), GADD153 (GeneTex, GTX11419, MW 31kDa), Caspase-8 (cell signaling #9429, MW 18kDa), ERK(cell signaling #4695, MW 44, 42kDa), ERK(SANTA CRUZ, sc-94, MW 44kDa), p-ERK(Cell signaling #9910, MW 44, 42kDa), p38(Cell signaling #9212, MW 43kDa), p-p38(Cell signaling #9910, MW 40kDa), p-p38(cell signaling #4511,MW 43kDa), JNK(Cell signaling #9252, MW 46kDa), p-JNK(Cell signaling #9910, MW 46kDa), p21(cell signaling #2947, MW 21kDa), and Caspase-3 (cell signaling #9661, MW 17kDa) were all obtained from Cell Signaling Technology Inc. (Beverly, MA, USA); p53 (Novus, NB100-92601, MW 43kDa) and JNK (Novus, NB100-192, MW 42kDa) antibodies were obtained from Novus Biologicals (Littleton, CO, USA). cdc2 (Santa Cruz, sc-54, MW 34kDa), cyclin-A (Santa Cruz, sc-751, MW 54kDa), cyclin-B1 (Santa Cruz, sc-245, MW 60kDa) antibodies were obtained from Santa Cruz Biotechnology, Inc. (Dallas, Texas, USA).

Cell culture. AGS cells were placed into 75-cm^2 tissue culture flasks and maintained in F-12K contained with 10% heat-inactivated fetal bovine serum (FBS) (Gibco BRL), 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were grown at 37˚C in a humidified atmosphere of 95% air and 5% CO_2. All data presented are from at least three independent experiments.

Cytotoxicity assay. The cytotoxicity of Tan-IIA towards AGS cells was evaluated by MTT assay in triplicate as described elsewhere (13). Briefly, The AGS cells were plated in 96-well plates at a density of 2×10^4 cells/well for 16-20 h. Thereafter, the cells were treated with different concentrations (0, 1, 3, 9, 15, 30 and 60 μg/ml) of Tan-IIA for 24, 48 and 72 h. Subsequently, the cells were incubated with 1 mg/ml of MTT in fresh complete F-12K medium for 1 h. The surviving cells converted MTT to formazan by forming a blue-purple color when dissolved in dimethyl sulfoxide. The intensity of formazan was measured at 590 nm using a microplate reader. The relative percentage of cell viability was calculated by dividing the absorbance of treated cells by that of the untreated control in each experiment, using the following formula: proliferation rate (%)=(OD_{test}−OD_{blank}) ×100, where OD_{test} and OD_{blank} were the optical density of the test substances and the blank control, respectively.

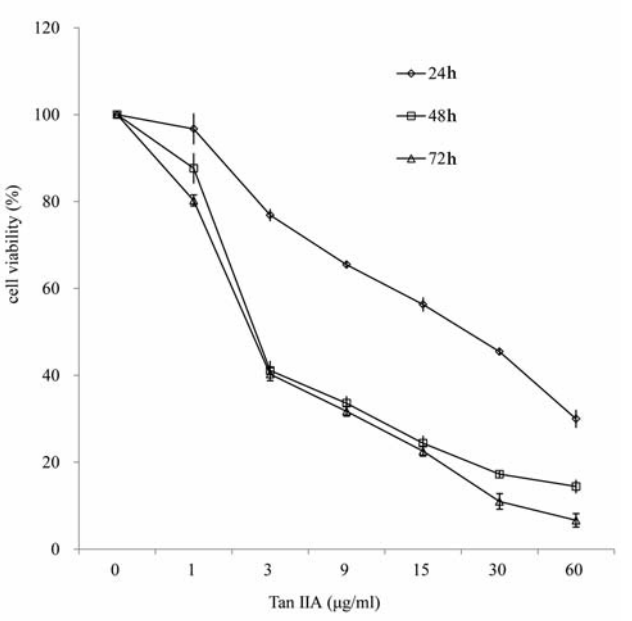
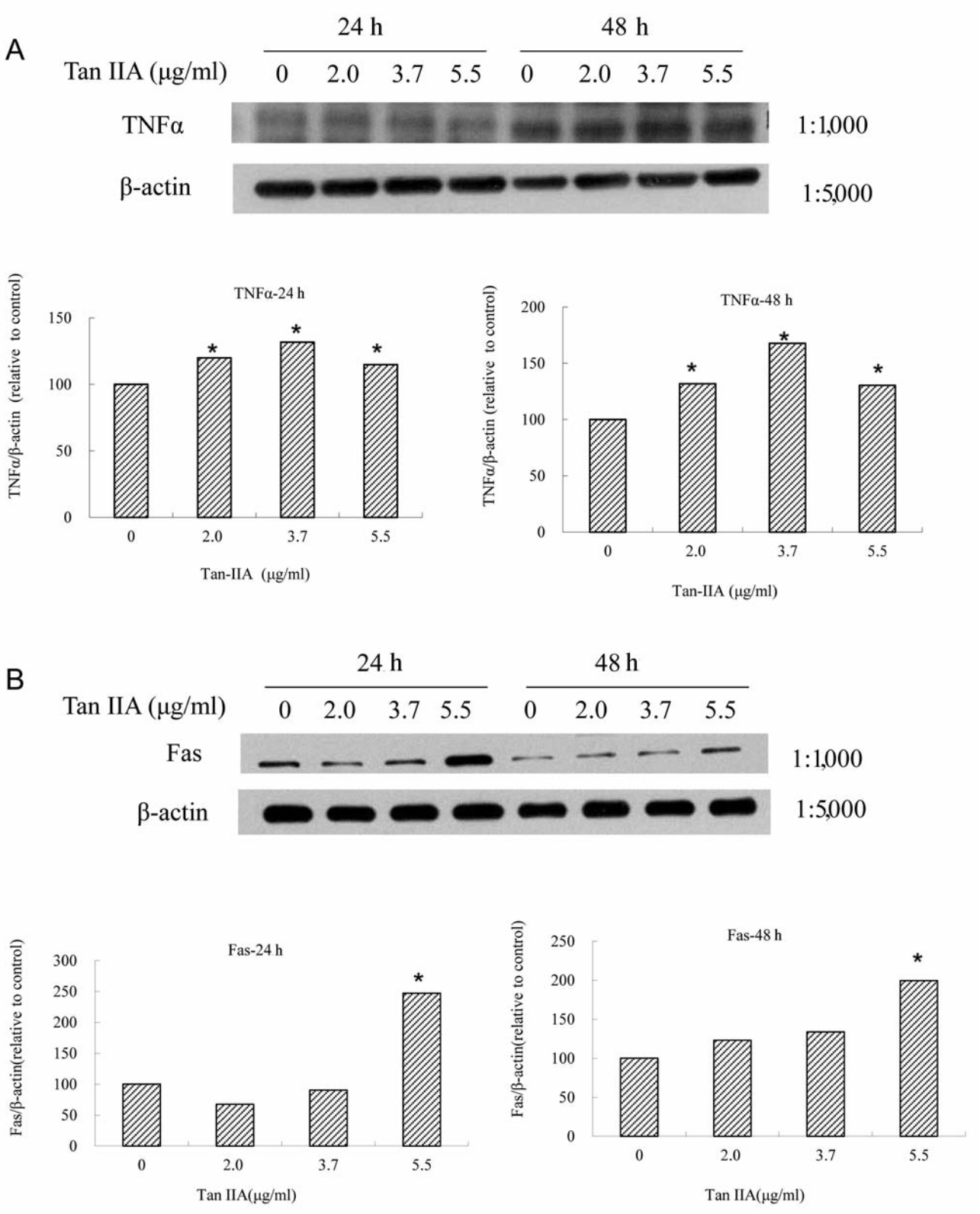


Figure 1. *Cytotoxic effects of Tanshinone IIA (Tan-IIA) on AGS cells. The cytotoxic effects of Tan-IIA on AGS cells were determined by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay as described in the Materials and Methods. Each data point is the mean±SD of the experiments.*

Western blot analysis. The western blot procedures were performed as described elsewhere (14, 15). Briefly, AGS cells were treated with affront concentrations of Tan-IIA for different durations, and then the cells were lysed in the ice-cold whole cell extract buffer containing protease inhibitors. The lysate was vibrated for 30 minutes at 4˚C and centrifuged at 12,281 × *g* for 10 min. Protein concentration was then measured by BCA protein assay kit (Pierce, Rockford, IL, USA). Equal amounts of proteins were subjected to electrophoresis using 12% sodium dodecyl sulfate-polyacrylamide gels. To verify equal protein loading and transfer, proteins were then transferred to polyvinylidene difluoride membranes and the membranes were blocked for 1 h at 4˚C using blocking buffer [5% non-fat dried milk in solution containing 50 mM Tris/HCl (pH 8.0), 2 mM $CaCl_2$, 80 mM sodium chloride, 0.05% Tween 20 and 0.02% sodium azide). The membranes were then incubated for 2 h at room temperature with specific primary antibody followed by anti-rabbit or anti-mouse immunoglobulin G-horseradish peroxidase-conjugated secondary antibodies. The membranes were washed three times for 10 min with washing solution. Finally, the protein bands were visualized on X-ray film using the enhanced chemiluminescence detection system (PerkinElmer Life and Analytical Sciences, Boston, MA, USA) and quantification process by using image J.

Cell-cycle analysis by flow cytometric assay of AGS cells treated with Tan-IIA. The cell-cycle distribution was determined by flow



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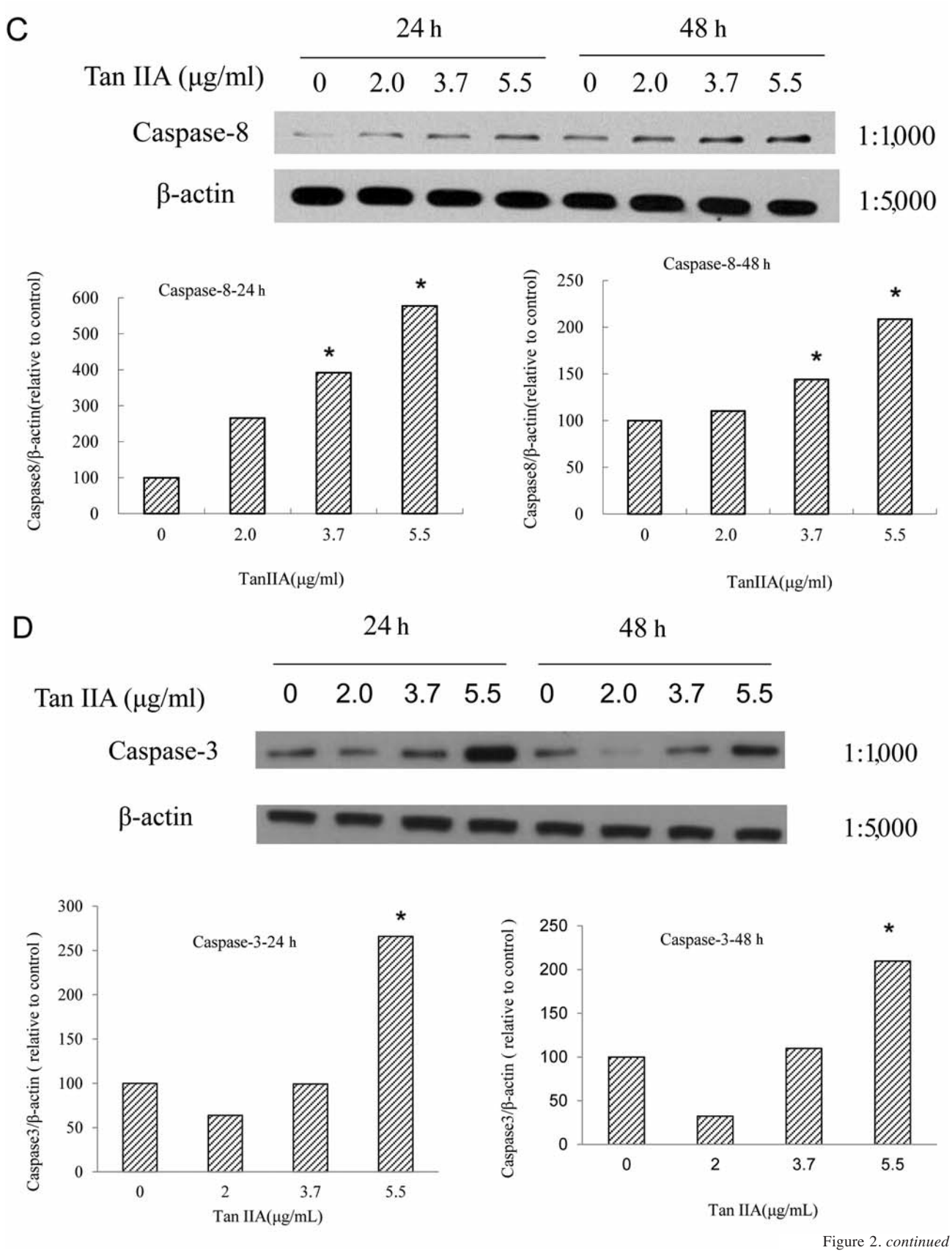

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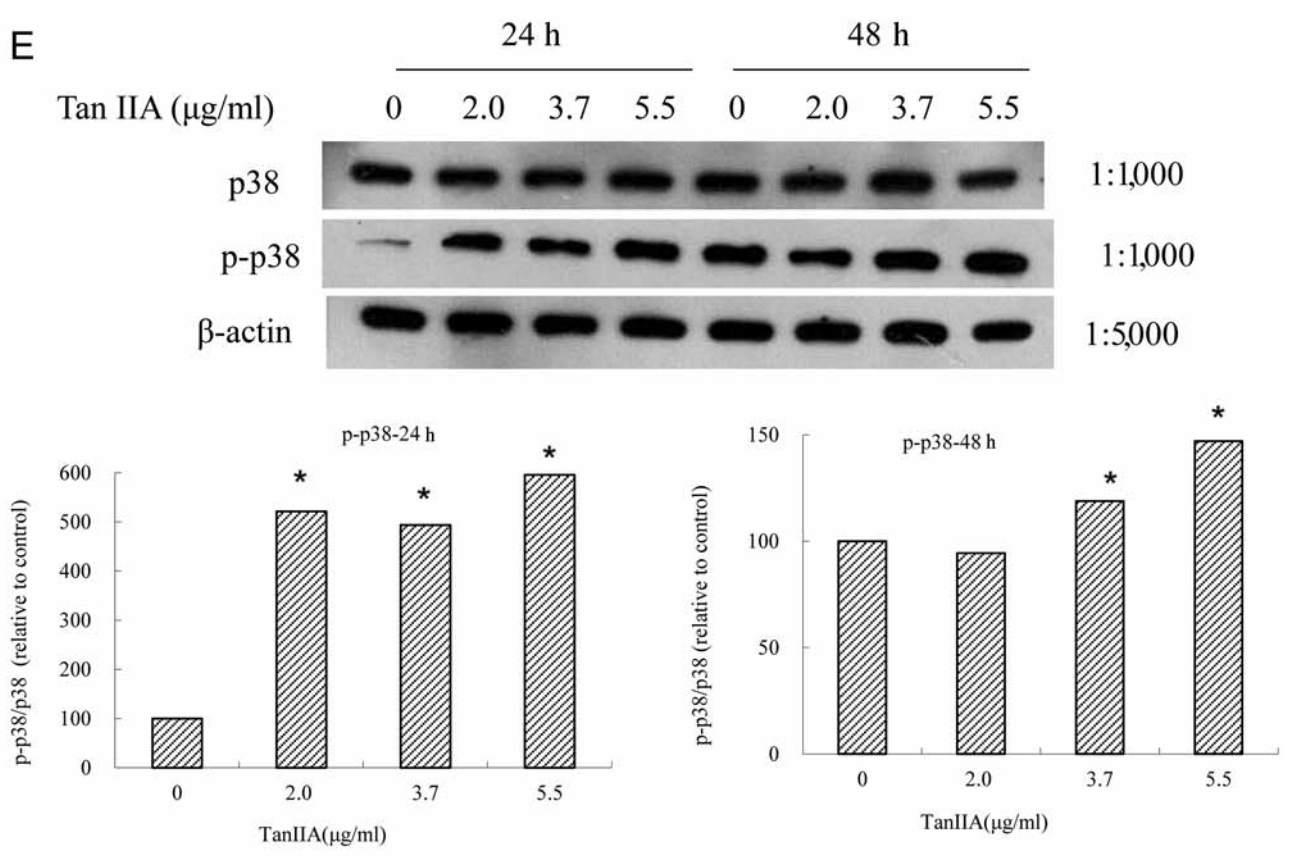

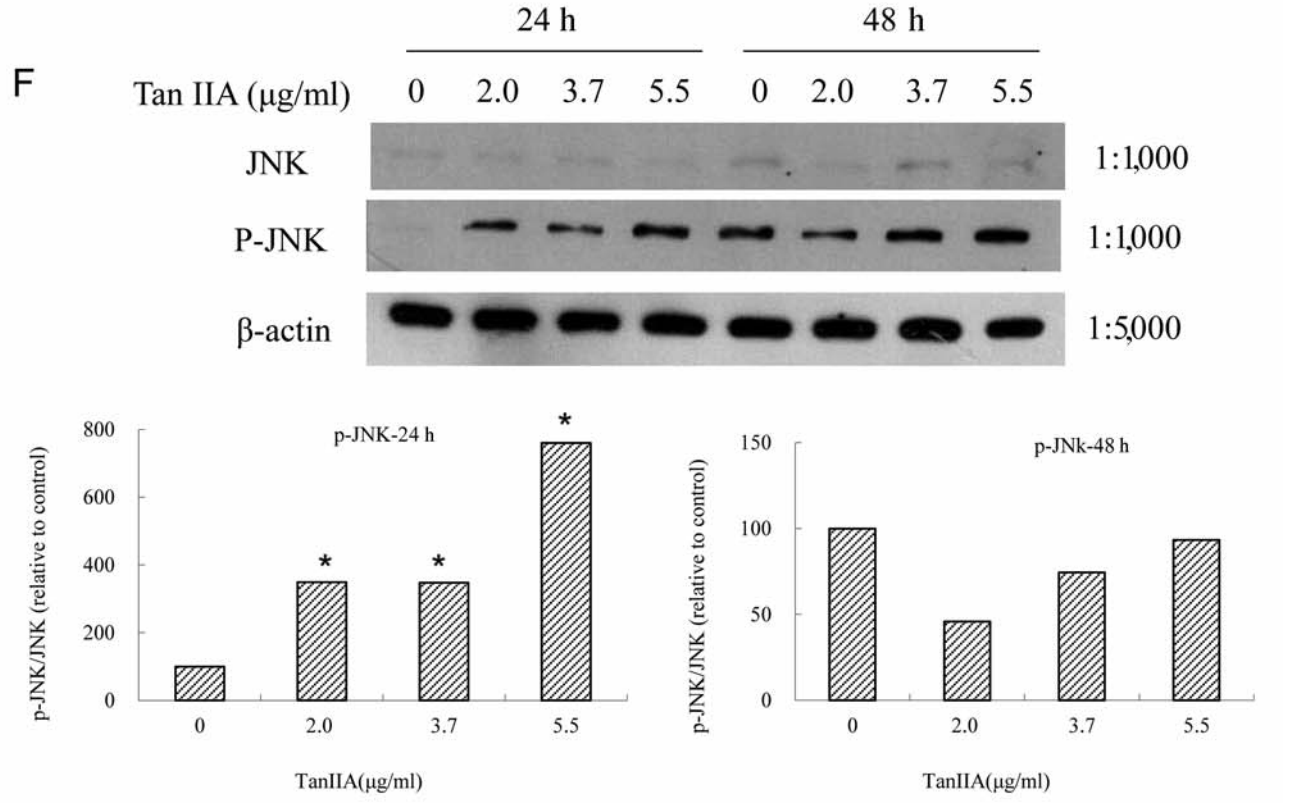

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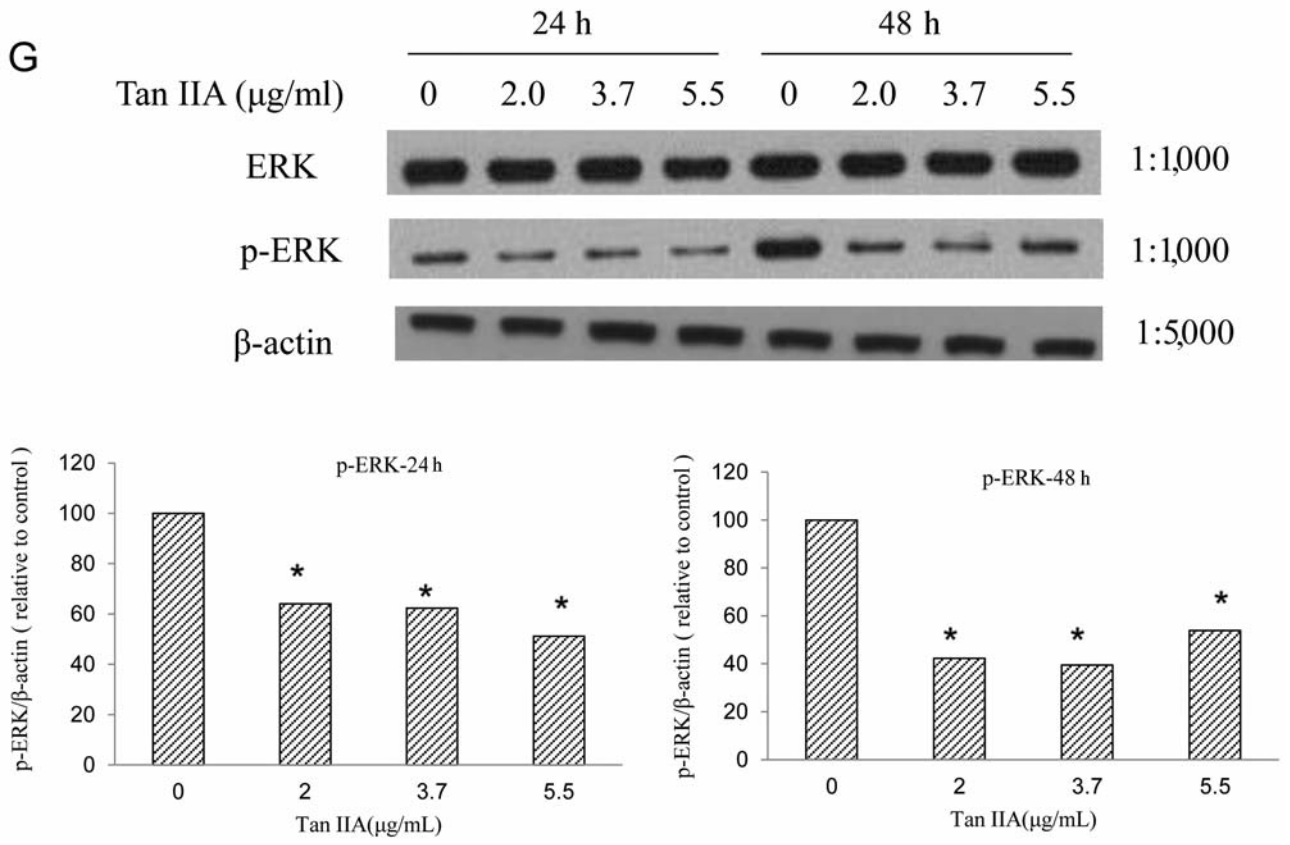


Figure 2. *Protein expression of tumor necrosis factor-alpha (TNFα), FAS, caspase-8, caspase-3, extracellular-related kinase (ERK), phospho extracellular-related kinase (p-ERK), p38, p-p38, Jun-amino-terminal kinase (JNK), phospho Jun-amino-terminal kinase (p-JNK) and β-actin in AGS cells. AGS cells were treated with different concentrations of Tan-IIA (0, 2.0, 3.7 and 5.5 μg/ml) for 24 48 h and then the protein expression levels were evaluated by western blot analysis as described in the Materials and Methods. The results revealed that Tan-IIA dose-dependently increased the protein expression of TNFα (A), FAS (B), caspase-8 (C), caspase-3 (D), p-p38(E) and p-JNK (F) but decreased p-ERK (G) level significantly.*

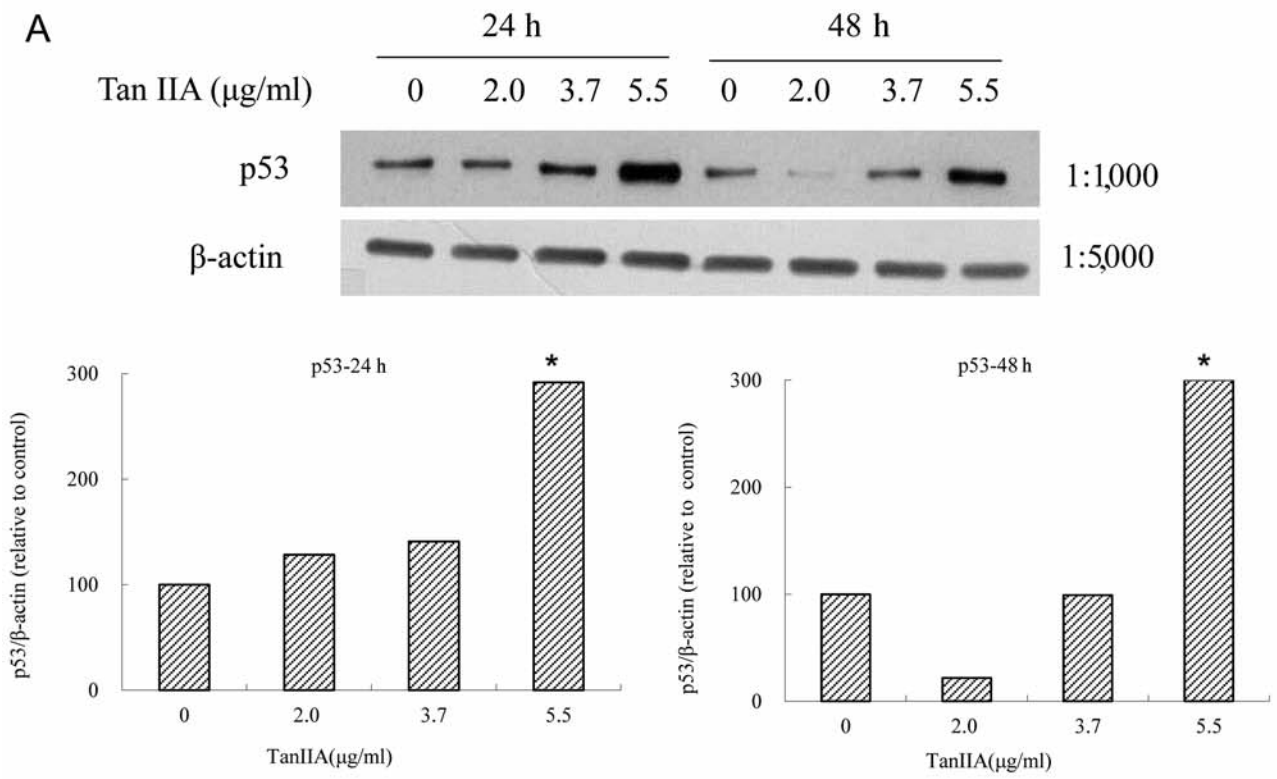
cytometry as described previously (16). Briefly, AGS cells (1×10^6/6 cm Petri dish) in complete medium for 16-20 h, then were incubated with different concentrations of Tan-IIA (0, 2.0, 3.7 and 5.5 μg/ml) for 24 or 48 h before cells were harvested by centrifugation. After being harvested, cells were washed with PBS, then fixed gently (drop by drop) in 70% ethanol (in PBS) overnight at −20˚C and resuspended in PBS containing 10 μg/ml PI and 20 μg/ml RNase (Sigma) for 45 min at 37˚C in the dark. Cells were then analyzed in a CytomicsTM FC500 Flow Cytometer (Beckman Coulter, San Jose, CA, USA) equipped with an argon laser at 488 nm. A minimum of 10,000 cells was analyzed for DNA content, and the percentage of cells in each cell cycle phase was quantified. Cell cycle distribution and apoptosis were determined and analyzed using Cytomics CXP Analysis (Beckman Coulter, San Jose, CA, USA). The average percentage of cells in each phase of the cell cycle was determined from three independent experiments.

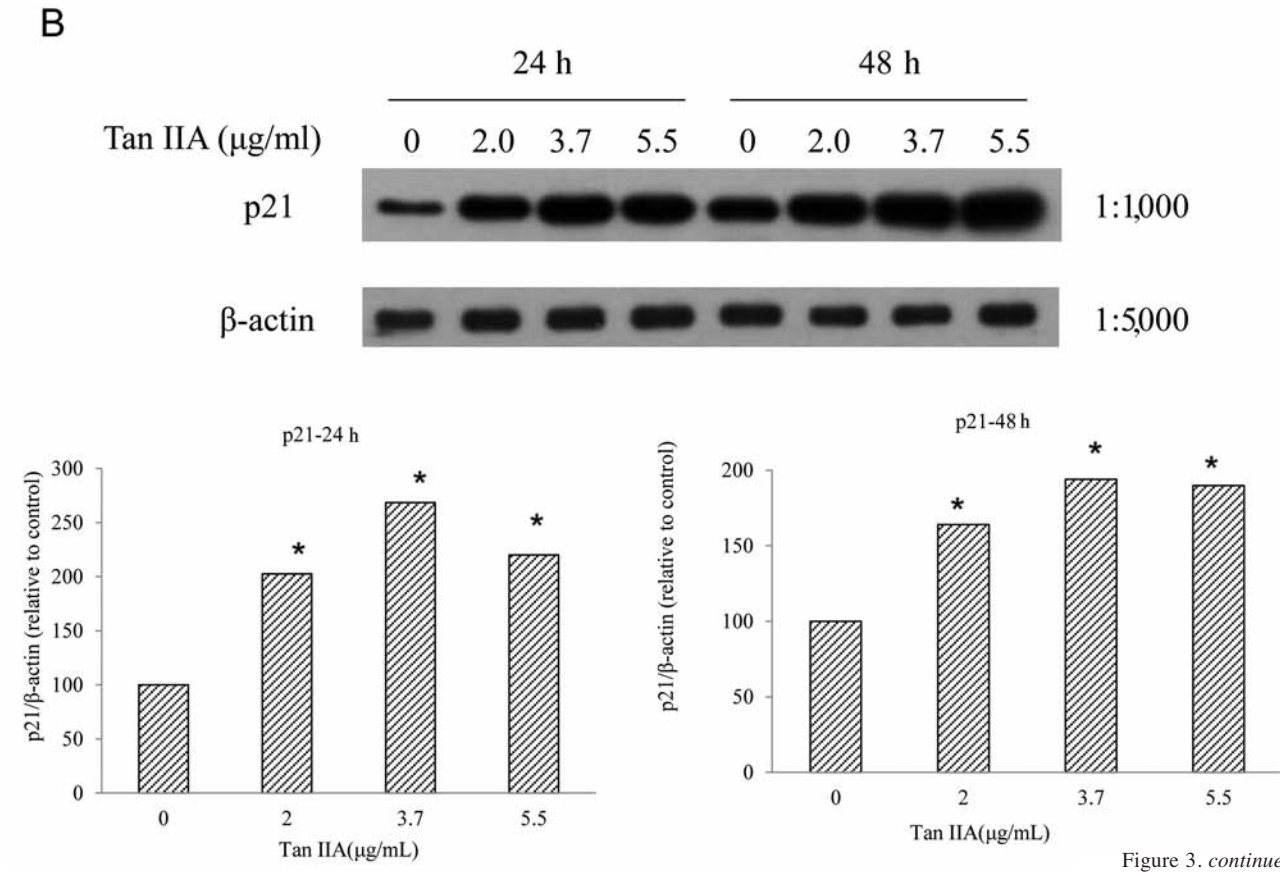
Statistical analysis. Values are presented as the means±SD. Student's *t*-test was used to analyze statistical significance. A *p*-value of less than 0.05 was considered to indicate a statistically significant difference for all the tests.

Results

Effects of Tan-IIA on the viability of AGS cells. The results revealed that Tan-IIA inhibited AGS cells in a time- and dose-dependent manner. The half-maximal inhibitory concentration (IC_{50}) was 5.5, 3.7 and 3.5 μg/ml at 24, 48 and 72 h, respectively (Figure 1).

Concentration-dependent effects of Tan-IIA on the protein expression of TNFα, FAS, caspase-3, caspase-8, ERK, p-ERK, p38, p-p38, JNK, p-JNK, p53, p21, CDC2, cyclin A and cyclin B1 in AGS cells. AGS cells were treated with different concentrations of Tan-IIA for 24 or 48 h and then the protein expression of TNFα, FAS, caspase-3, caspase-8, ERK, p-ERK, p38, p-p38, JNK, p-JNK, p53, p21, CDC2, cyclin A, cyclin B1 and β-actin were evaluated by western blot analysis. The results showed that Tan-IIA increased the protein expression levels of TNFα, FAS, caspase-3, caspase-8, p-p38/p38 and





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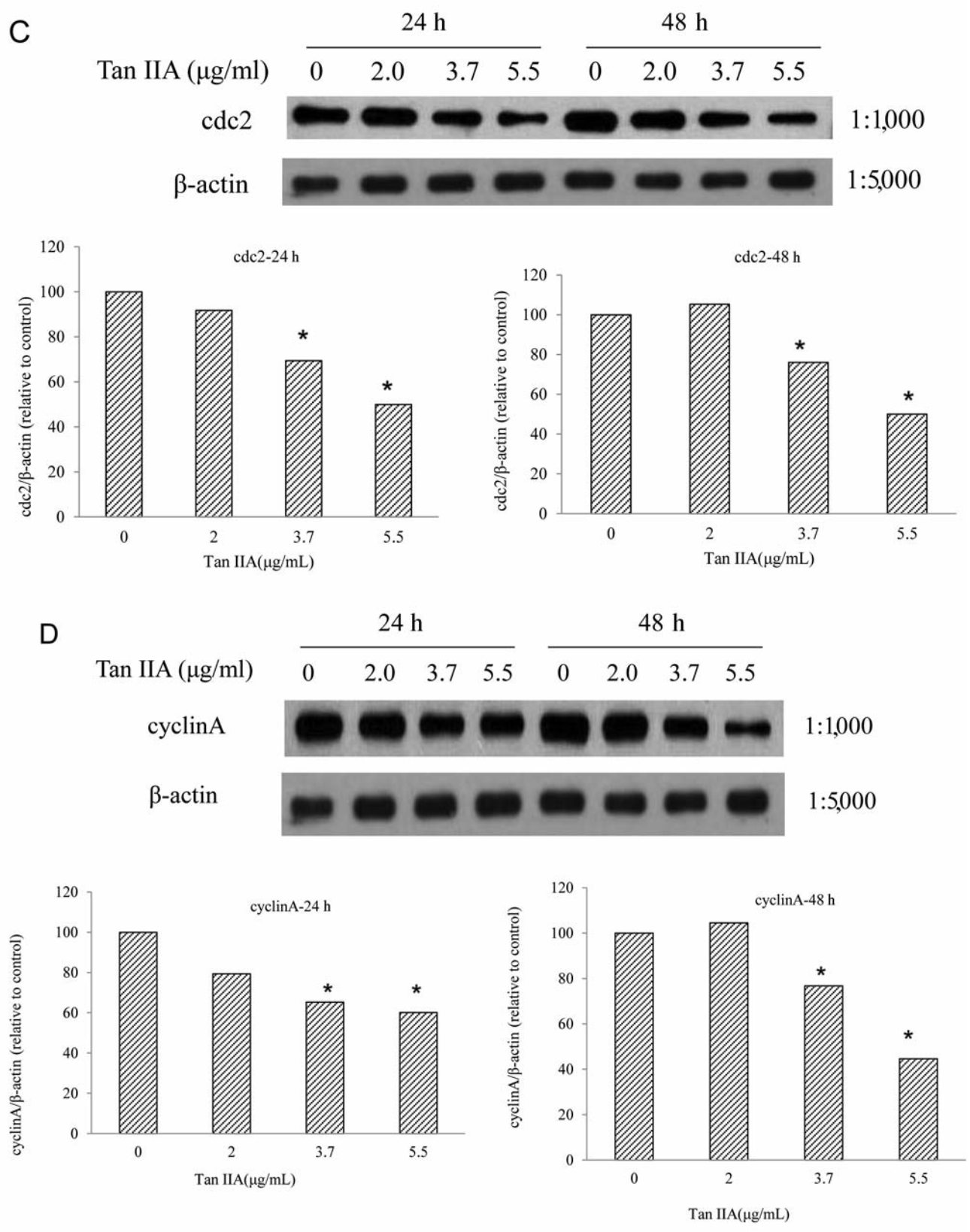

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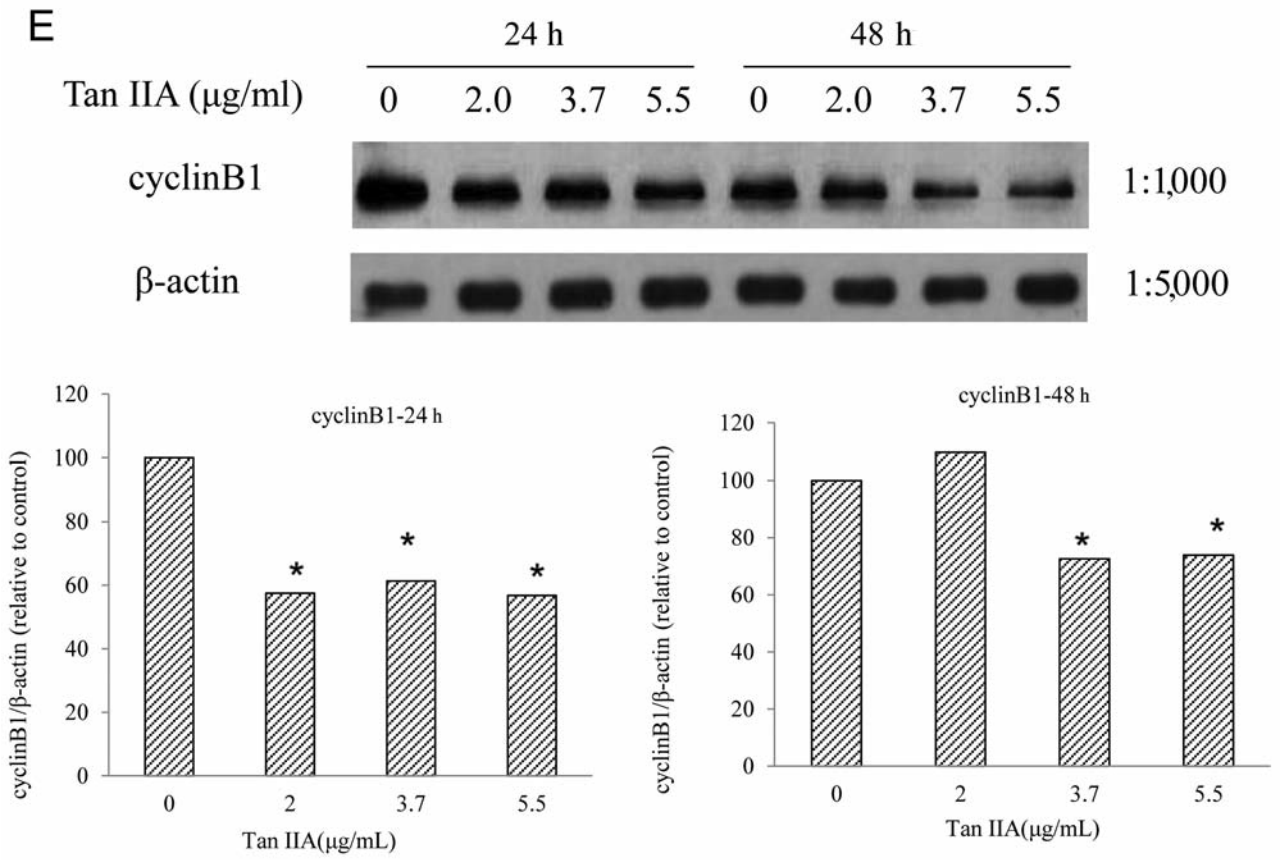


Figure 3. *Protein expressions of p53, p21, cell division cycle protein 2 (CDC2), cyclin A, cyclin B1 and β-actin in AGS cells. The AGS cells were treated with different concentrations of Tanshinone IIA (Tan-IIA) (0, 2.0, 3.7 and 5.5 μg/ml) for 24 or 48 h and then the protein expression levels were evaluated by western blot analysis, as described in the Materials and Methods. The results revealed that Tan-IIA can increase the protein expression level of p53 (A) and p21 (B), but reduces that of CDC2 (C), cyclin A (D) and cyclin B1 (E) dose-dependently.*

p-JNK/JNK (Figure 2 A-F, respectively), but reduced p-ERK and ERK (Figure 2G) levels significantly.

The results showed that Tan-IIA increased the protein expression of p53 and p21 (Figure 3A and B), but reduced levels of CDC2, cyclin A and cyclin B1 (Figure 3 C-E) significantly.

Time-dependent effects of Tan-IIA on protein expression in AGS cells. AGS cells were treated with Tan-IIA (3.7 μg/ml) for different durations (0, 24 and 48 h) and then the protein expression levels of TNFα, FAS, caspase-3, caspase-8, ERK, p-ERK, p38, p-p38, JNK, p-JNK, p53, p21,CDC2, cyclin A, cyclin B1 and β-actin were evaluated by western blot analysis. The results showed that Tan-IIA increased the protein expression levels of TNFα, FAS, caspase-8, caspase-3, p-p38/p38 and p-JNK/JNK (Figure 4 A-F), and of p53 and p21 (Figure 5A and B, respectively), but significantly reduced that of p-ERK/ERK (Figure 4G) and CDC2, cyclin A and cyclin B1 (Figure 5C-E).

Effects of Tan-IIA on cell-cycle distribution. Cell-cycle distribution was analyzed by flow cytometry. The results showed that when AGS cells were treated with different concentrations of Tan-IIA for 24 and 48 h, cells in the G_2/M phase increased from 27.5% to 29.9%, 44.0% and 45.5% at 24 h; and from 29.7% to 28.2%, 48.0% and 48.5% at 48 h, respectively when when treated with 0, 2.0, 3.7 and 5.5 μg/ml, respectively (Figure 6). Results showed that treatment of AGS cells with Tan-IIA can induce G2/M phase arrest.

Discussion

The results showed that Tan-IIA can inhibit AGS human gastric cancer cells in a time- and dose-dependent manner *in vitro*. This is agreement with other documents (7-9).

It is well documented that the entry of all eukaryotic cells into mitosis is regulated by activation of CDC2/CDK1 at the G_2/M transition, begins with the binding of cyclin B1 to CDC2/CDK1 to form the mitosis-promoting factor (17, 18).

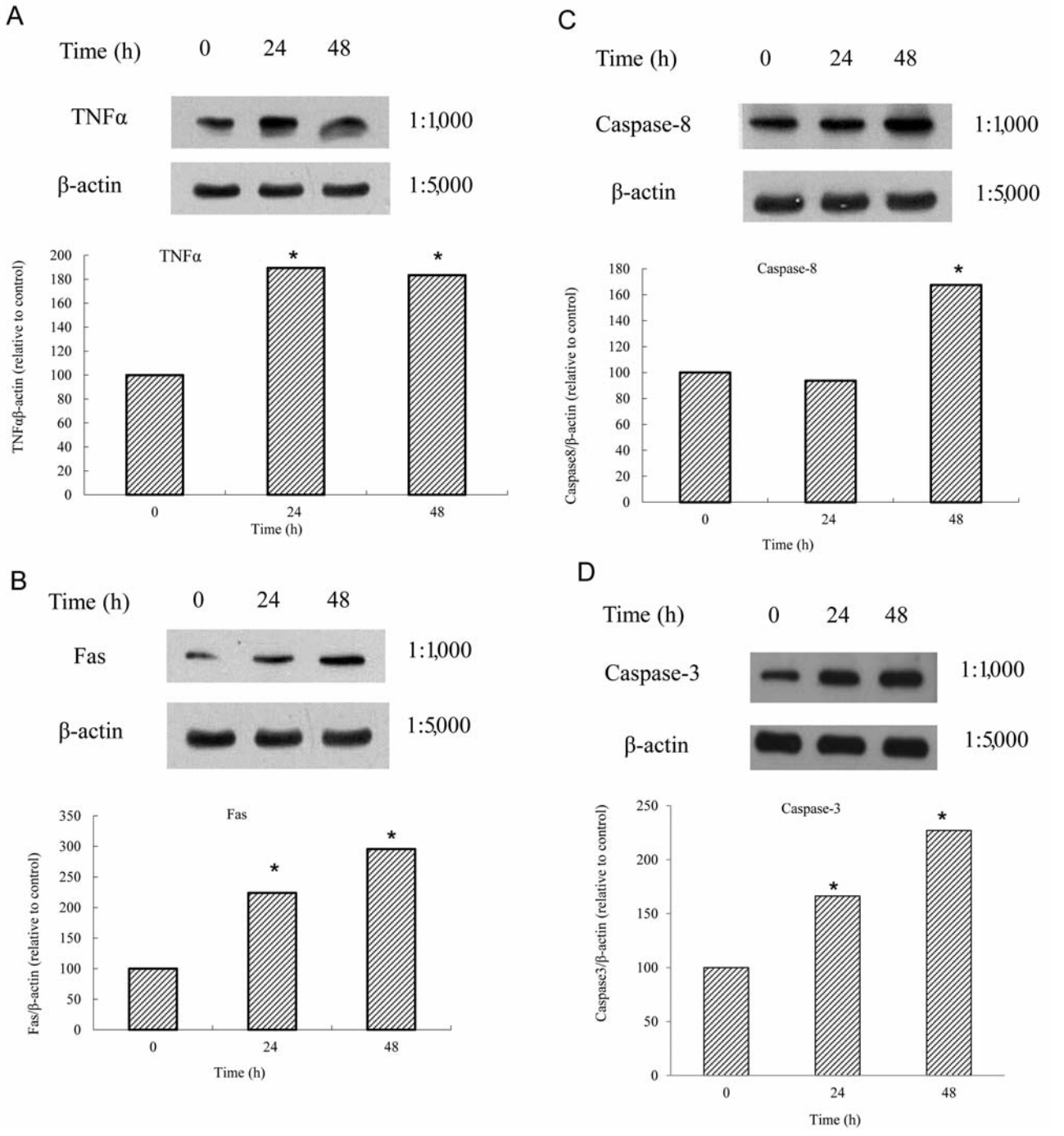


Figure 4. *continued*

Junttila *et al*. documented that the ERK pathway can also be negatively regulated by the p38-JNK pathways to promote apoptosis (19). Nordstrom *et al*. also showed that the ERK and the p38-JNK MAPK pathways appear to exert opposing effects (20). ERK, p38, and JNKs belong to the MAPK pathway. These kinases are activated *via* phosphorylation in response to cytokines, growth factors and stress. p-ERK can inhibit apoptosis but p-p38 can induce apoptosis (21-23).

Our results demonstrated that cells treated with Tan-IIA can reduce the protein expression level of p-ERK, but increase p-p38 and p-JNK levels in AGS cells in time- and dose-dependent manner. Our results also demonstrated that

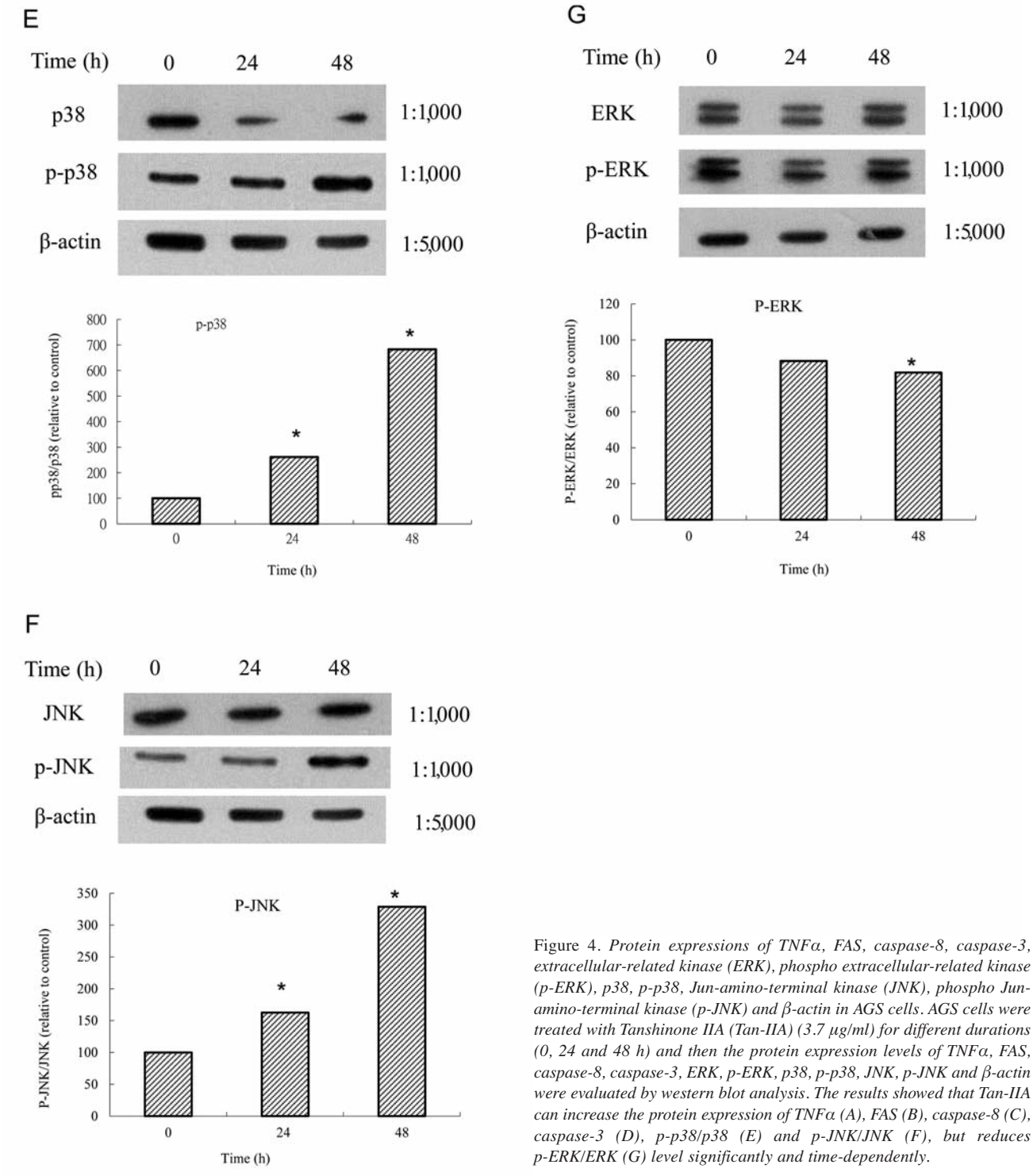
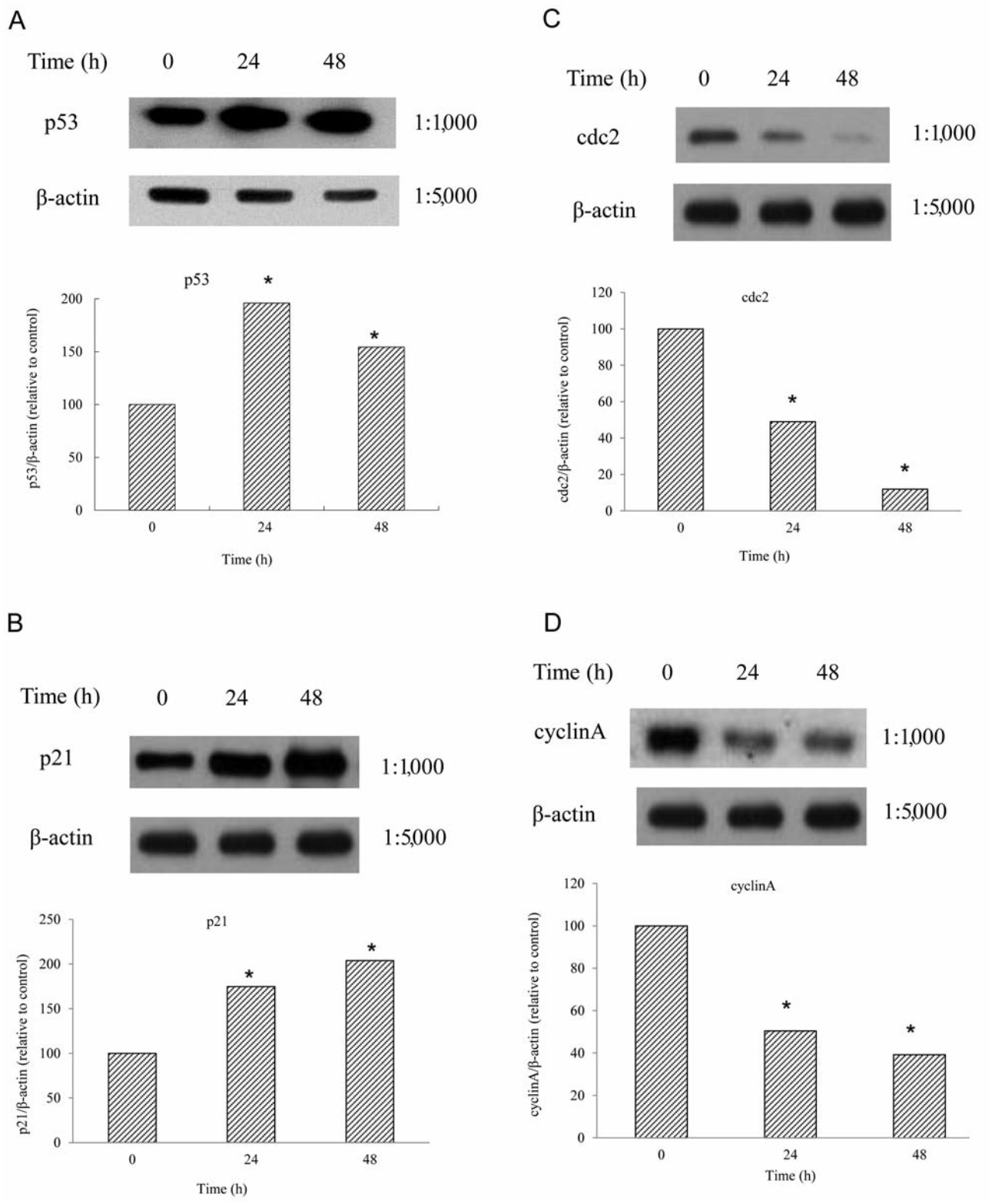


Figure 4. *Protein expressions of TNFα, FAS, caspase-8, caspase-3, extracellular-related kinase (ERK), phospho extracellular-related kinase (p-ERK), p38, p-p38, Jun-amino-terminal kinase (JNK), phospho Jun-amino-terminal kinase (p-JNK) and β-actin in AGS cells. AGS cells were treated with Tanshinone IIA (Tan-IIA) (3.7 μg/ml) for different durations (0, 24 and 48 h) and then the protein expression levels of TNFα, FAS, caspase-8, caspase-3, ERK, p-ERK, p38, p-p38, JNK, p-JNK and β-actin were evaluated by western blot analysis. The results showed that Tan-IIA can increase the protein expression of TNFα (A), FAS (B), caspase-8 (C), caspase-3 (D), p-p38/p38 (E) and p-JNK/JNK (F), but reduces p-ERK/ERK (G) level significantly and time-dependently.*

the treatment of AGS cells with Tan-IIA can increase the protein expression of p53 and p21, but reduce that of CDC2, cyclin B1 and cyclin A to induce G_2/M arrest. This is agreement with Dong *et al*. who showed Tan-IIA up-regulated the expression of p53 gene and induced G2/M phase arrest in MKN-45 gastric cancer cells (9).

It is well-documented that TNFα can induce moderate response of the p38-MAPK pathway, leading to strong



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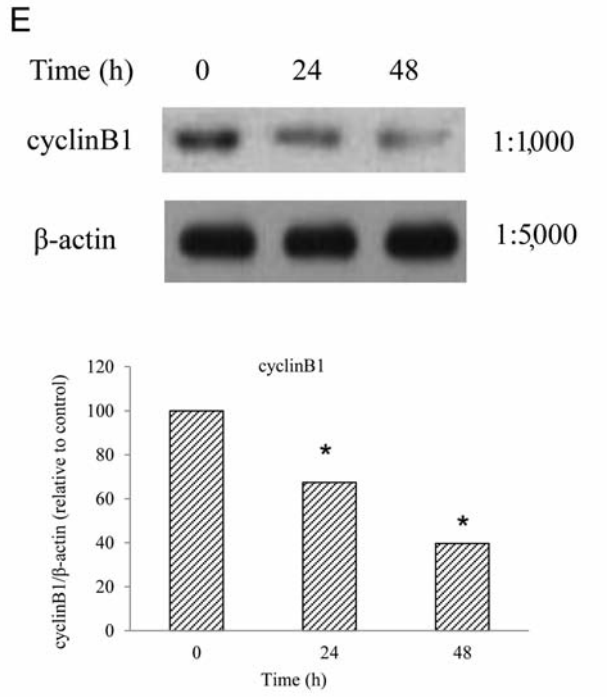

Figure 5. *Effects of Tanshinone IIA (Tan-IIA) on the protein expression of p53, p21, CDC2, cyclin A, cyclin B1 and β-actin in AGS cells. AGS cells were treated with Tan-IIA (3.7 μg/ml) for different durations (0, 24 and 48 h) and then the protein expression levels of p53, p21, CDC2, cyclin A, cyclin B1 and β-actin were evaluated by western blot analysis. The results showed that Tan-IIA can increase the protein expression of p53 (A) and p21 (B), but reduces that of CDC2 (C), cyclin A (D) and cyclin B1 (E) significantly and time dependently.*

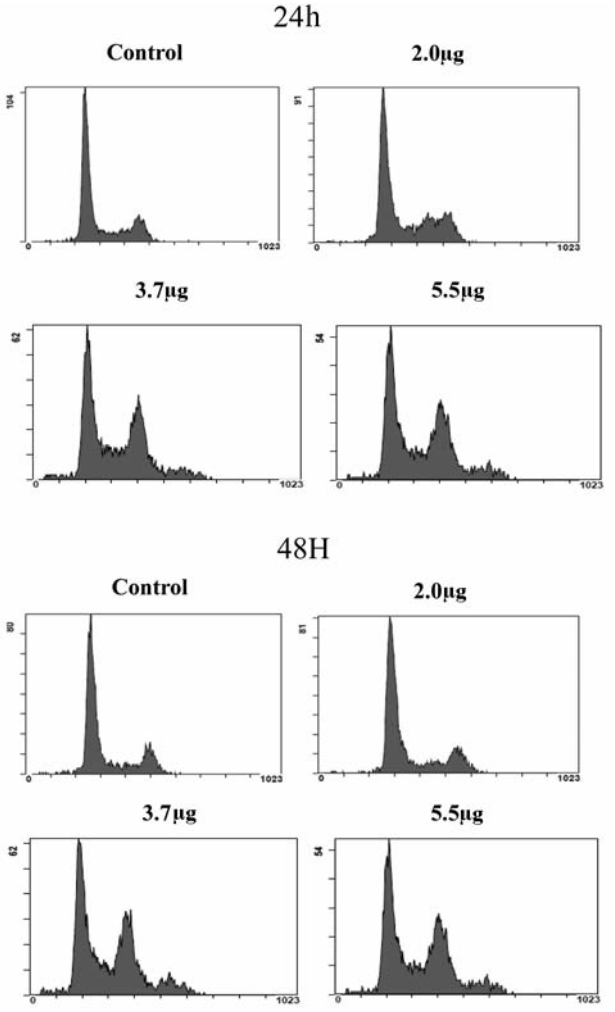

Figure 6. *Effects of Tanshinone IIA (Tan-IIA) on the cell cycle. AGS cells were treated with Tan-IIA (0, 2.0, 3.7 and 5.5 μg/ml) for 24 or 48 h, and the cell-cycle distribution was analyzed by flow cytometry, as described in the Materials and Methods. The results showed that the percentage of AGS cells in the G_2/M phase increased from 27.5% to 29.9%, 44.0% and 45.5% at 24 h, and from 29.7% to 28.2%, 48.0% and 48.5% at 48 h, respectively.*

activation of the stress-related JNK group (24). TNF also can induce cell death, bind to TNF receptor type 1, resulting in the activation of caspase-8, thus inducing apoptosis (25). The FAS receptor is a death receptor that leads to apoptosis (26). Our results also showed that treatment with Tan-IIA can increase the protein expression levels of TNFα, FAS, caspase-8 and caspase-3 in AGS cells. These findings indicate that one of the molecular mechanisms of action of Tan-IIA involved in inhibition of AGS cell proliferation may be through the extrinsic apoptotic pathway. Another molecular mechanism may be through increasing the protein expression of p-p38 and p-JNK, but reducing p-ERK to induce the activation of p53, followed by increasing the protein expression of p21 to down-regulate CDC2 and cyclin B1 expression and then to induce G_2/M phase arrest.

The proposed model for inhibition of the proliferation of AGS cells by Tan-IIA is shown in Figure 7. To our knowledge, this is the first report that Tan-IIA can inhibit AGS human gastric cancer cells through the extrinsic and MAPK pathway. The chemotherapeutic potential of Tan-IIA for human gastric cancer warrants further study.

Acknowledgements

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References

1 Che AJ, Zhang JY, Li CH, Chen XF, Hu ZD and Chen XG: Separation and determination of active components in radix *Salviae miltiorrhizae* and its medicinal preparations by nonaqueous capillary electrophoresis. J Sep Sci *27*: 569-575, 2004.

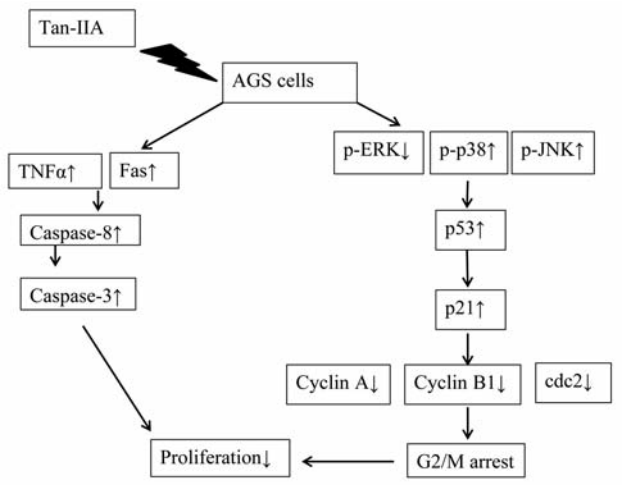

Figure 7. *The proposed model for Tanshinone IIA (Tan-IIA) inhibition of the AGS cell proliferation. Tan-IIA inhibits AGS human gastric cancer cells through extrinsic pathway and mitogen-activated protein kinases (MAPK) pathway.*

2 Zhou L, Zuo Z and Chow MS: Danshen: An overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J Clin Pharmacol *45*: 1345-1359, 2005.

3 Su CC, Chen GW, Kang JC and Chan MH: Growth inhibition and apoptosis induction by tanshinone IIA in human colon adenocarcinoma cells. Planta Med *74*: 1357-1362, 2008.

4 Su CC and Lin YH: Tanshinone IIA inhibits human breast cancer cells through increased Bax to Bcl-xL ratios. Int J Mol Med *22*: 357-361, 2008.

5 Chiu TL and Su CC: Tanshinone IIA induces apoptosis in human lung cancer A549 cells through the induction of reactive oxygen species and decreasing the mitochondrial membrane potential. Int J Mol Med *25*: 231-236, 2010.

6 Hou J, He J, Jin X, Hu T and Zhang Y: Study on optimisation of extraction process of tanshinone IIA and its mechanism of induction of gastric cancer SGC7901 cell apoptosis. Afr J Tradit Complement Altern Med *10*: 456-458, 2013.

7 Xu M, Cao FL, Li NY, Liu YQ, Li YP and Lv CL: Tanshinone IIA reverses the malignant phenotype of SGC7901 gastric cancer cells. Asian Pac J Cancer Prev *14*: 173-177, 2013.

8 Chen J, Shi DY, Liu SL and Zhong L: Tanshinone IIA induces growth inhibition and apoptosis in gastric cancer *in vitro* and *in vivo*. Oncol Rep *27*: 523-528, 2012.

9 Dong X, Dong J and Peng G: Growth-inhibiting and apoptosis-inducing effects of Tanshinone II A on human gastric carcinoma cells. J Huazhong Univ Sci Technolog Med Sci *27*: 706-709, 2007.

10 Carswell EA, Old LJ, Kassel RL, Green S, Fiore N and Williamson B: An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci USA *72*: 3666-3670, 1975.

11 Gaur U and Aggarwal BB: Regulation of proliferation, survival and apoptosis by members of the TNF superfamily. Biochem Pharmacol *66*: 1403-1408, 2003.

12 Molina JR and Adjei AA: The RAS/RAF/MAPK Pathway. J Thorac Oncol *1*: 7-9, 2006.

13 Mossman T: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J Immunol Methods. 65: 55-63, 1983.

14 Bradford MM: A rapid and sensitive method for the quantization of microgram quantities of protein using the principle of protein-dye binding, Anal Biochem *72*: 248-254,1976.

15 Chen HC, Hsieh WT, Chang WC and Chung JG: Aloe-emodin induced *in vitro* G_2/M arrest of cell cycle in human promyelocytic leukemia HL-60 cells, Food Chem Toxicol *42*: 1251-1257, 2004.

16 Li TM, Chen GW, Su CC, Lin JG, Yeh CC, Cheng KC and Chung JG: Ellagic acid induced p53/p21 expression, G1 arrest and apoptosis in human bladder cancer T24 cells. Anticancer Res *25*: 971-979, 2005.

17 Lorca T, Labbé JC, Devault A, Fesquet D, Capony JP, Cavadore JC, Le Bouffant F and Dorée M: Dephosphorylation of cdc2 on threonine 161 is required for cdc2 kinase inactivation and normal anaphase. EMBO J *11*: 2381-2390, 1992.

18 Wade Harper J and Stephen J: Elledge The role of Cdk7 in CAK function, a retro-retrospective Genes Dev *12*: 285-289, 1998.

19 Junttila MR, Li SP and Westermarck J: Phosphatase-mediated crosstalk between MAPK signaling pathways in the regulation of cell survival. FASEB J *22*: 954-965, 2007.

20 Nordström E, Fisone G and Kristensson K: Opposing effects of ERK and p38-JNK MAP kinase pathways on formation of prions in GT1-1 cells. FASEB J *23*: 613-622, 2009.

21 Xia Z, Dickens M, Raingeaud J, Davis RJ and Greenberg ME: Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science *270*: 1326-1331, 1995.

22 Rubinfeld H and Seger R: The ERK cascade: a prototype of MAPK signaling. Mol Biotechnol *31*: 151-174, 2005.

23 Wagne EF and Nebreda AR: Signal integration by JNK and p38 MAPK pathways in cancer development. Nat Rev Cancer *9*: 537-549, 2009.

24 Kant S, Swat W, Zhang S, Zhang ZY, Neel BG, Flavell RA and Davis RJ: TNF-stimulated MAP kinase activation mediated by a Rho family GTPase signaling pathway. Genes Dev *25*: 2069-2078, 2011.

25 Gaur U and Aggarwal BB: Regulation of proliferation, survival and apoptosis by members of the TNF superfamily. Biochem. Pharmacol *66*: 1403-1408, 2003.

26 Wajant H: The Fas signaling pathway: more than a paradigm. Science *296*: 1635-1636, 2002.

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