# Chronic restraint stress promotes gastric epithelial malignant transformation by activating the Akt/p53 signaling pathway via ADRB2

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**Abstract.** The etiology of gastric cancer is associated with infectious, environmental and dietary factors, as well as genetic background. Additionally, emerging evidence has supported the vital role of chronic emotional stress on gastric carcinogenesis; however, the underlying mechanism remains unclear. The present study aimed to investigate the effects of chronic stress and a detrimental diet on gastric malignant epithelial transformation in rats. Therefore, 26 Wistar rats were randomly divided into the following four groups: i) Control; ii) detrimental diet (DD); iii) detrimental diet with chronic restraint (DR) and iv) detrimental diet with chronic restraint and propranolol treatment (DRP). ELISA was performed to detect the serum levels of epinephrine and norepinephrine. Epithelial cell apoptosis was analyzed using the TUNEL assay. The mRNA and protein expression levels of Akt and p53 were detected using reverse transcription quantitative PCR and western blotting, respectively. Pathological changes were analyzed using hematoxylin and eosin staining (H&E). The H&E staining results showed that dysplasia in the gastric mucosa occurred in two of eight rats in the DD group and in four of five rats in the DR group, whereas no dysplasia was detected in the DRP group. The apoptotic ratios of gastric epithelial cells were significantly decreased in all treatment groups compared with the control group. Adrenoceptor β2 (ADRB2) protein expression levels were increased significantly only in the DR group and this effect was significantly reduced in the DRP group. The mRNA expression levels of Akt and p53 were significantly upregulated in the DD group, and Akt mRNA expression was further elevated in the DR group. With regard to protein expression, the levels of Akt and p-Akt were significantly increased in the DR group, whereas these effects were reversed in the DRP group. Furthermore, the ratio of p-p53/p53 protein was significantly reduced in the DD or DR groups, but was reversed in the DRP group. Collectively, the findings of the present study suggested that chronic restraint stress potentially aggravates the gastric epithelial malignant transformation induced by a detrimental diet, at least partially via the Akt/p53 signaling pathway mediated via ADRB2.

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*Abbreviations:* RT-qPCR, reverse transcription-quantitative PCR; ADRB2, adrenoceptor β2; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine

*Key words:* chronic restraint stress, epithelial malignant transformation, detrimental diet, propranolol, apoptosis, adrenergic

## Introduction

Gastric cancer (GC) is the fifth most common malignant tumor (1) with a high mortality rate worldwide; according to the International Agency for Research on Cancer, there were an estimated 769,000 deaths from stomach cancer in 2020 worldwide (2). Gastric carcinogenesis has been ascribed to numerous etiological factors, including *Helicobacter pylori* infection (3-5), a detrimental environment and diet (6) and genetics (7,8). These factors may lead to DNA damage (9-11), oncogene activation, or the disruption of the dynamic balance between the proliferation and apoptosis of gastric epithelial cells (5,12).

p53, an important tumor suppressor, serves key roles in repairing damaged DNA, arresting cells in the G1 or G2/M phase, promoting the apoptosis of cells that cannot be repaired and avoiding the generation of malignant progeny (13,14). The activated oncogene Akt promotes the phosphorylation of murine double minute 2 (MDM2) and the ubiquitination degradation of p53 (15,16). The MDM2 protein is an E3 ubiquitin ligase that serves a central role in regulating the stability of p53. In addition, Akt mediates the phosphorylation of MDM2 at Ser166 and Ser186, thus contributing to the MDM2 protein-mediated ubiquitination and degradation of p53 (17,18). The Akt/p53 signaling pathway is closely associated with the progression and metastasis of numerous types of cancer (18-22).

In recent years, the effects of social psychological stress on the development of GC have received increased

attention (23,24). Chronic stress, common in modern life, may exert a wide range of negative effects on host immunity, metabolism and other physiological conditions (25,26). Gastric epithelial cells are regulated by a complex psycho-neuroendocrine network, which is constantly affected by various chronic stress factors (26,27). Chronic stress has been reported to be capable of promoting tumor progression and metastasis via the activation of the sympathetic nervous system (23,28,29), in which the adrenoceptor β2 (ADRB2) serves a crucial role. The β-adrenergic system regulates the biological behavior of tumors via various mechanisms, including via the promotion of cell proliferation, evasion from apoptosis, the induction of angiogenesis and the enhancement of cellular migration and metastasis (25,30). Stress, which exerts effects similar to those of ADRB2 agonists, significantly promotes the growth and metastasis of xenograft tumors, which can be blocked via ADRB2 knockout or the ADRB2 antagonist, propranolol (28,31-34). Using two human GC cell lines (MGC-803 and HGC-27), Lu *et al* (35) reported that isoprenaline induces the epithelial-mesenchymal transition via stimulation of the ADRB2/STAT3 signaling pathway. Shi *et al* (36) reported that ADRB2 signaling upregulates the expression of MMP-7, which demonstrates its involvement in the invasion and metastasis of GC. Moreover, the upregulation of ADRB2 has been reported to be positively associated with tumor size, histological grade, lymph node metastasis and clinical stage in human GC samples (23). However, the psychosocial side of the etiology of gastric carcinogenesis still lacks sufficient experimental and clinical evidence.

In a review written by Qiao *et al* (25), βadrenergic signaling was reported to activate the degradation of p53 and DNA damage through the ARRB1/PKA pathway. Tang *et al* (30) also reported that the activation of adrenergic receptors by norepinephrine was involved in PI3K/Akt signal transduction. Taking these points into consideration, it was hypothesized that chronic stress may promote the malignant transformation of the gastric epithelium by activating the Akt/p53 signaling pathway via ADRB2. Therefore, the present study aimed to design a rat model of chronic restraint stress with a detrimental diet in order to explore the effects of chronic stress on the Akt/p53 signaling pathway.

## Materials and methods

*Animals*. A total of 26 male specific pathogen-free (SPF) Wistar rats (age, 4 weeks; weight, 100±20 g) were purchased from Jinan Pengyue Experimental Animal Breeding Co., Ltd. and reared in the SPF facility at the Medical Experimental Animal Center of Weifang Medical University (Weifang, China) with 12 h light/dark cycles. The room temperature and relative humidity were maintained at 24±2˚C and 35-45%, respectively. All rats had free access to water and food (SPF grade); the food was purchased from Beijing KeaoXieli Feed Co., Ltd. (cat. no. 21123213). The present study was approved by the Animal Experimental Ethics Committee of Weifang Medical University (approval no. 2020SDL166).

*Experimental protocol*. All rats were fed adaptively for 2 weeks and then randomly divided into the following four treatment groups: i) Control (n=6); ii) detrimental diet (DD; n=8); iii) detrimental diet with chronic restraint (DR; n=5) and iv) detrimental diet with chronic restraint and propranolol treatment (DRP; n=7). The detrimental diet in this study referred to drinking water mixed with N-methyl-N'-nitro-N-nitrosoguanidine [MNNG (a DNA mutagenic agent); cat. no. C11744315; Shanghai Macklin Biochemical Co., Ltd.] and gavage with high salt (10% sodium chloride solution). All rats were fed a normal diet. The rats in the control group had free access to clean drinking water, whereas those in the other three treatment groups were free to drink water containing 167 $\mu$g/ml MNNG except for during the period of restraint. Besides the control group, 10% sodium chloride solution was administered to the rats via gavage at a dose of 5 ml/kg body weight once per day. Rats in the DR and DRP groups were treated under a standard restraint environment for 3 h/day for 8 weeks as previously described (37,38). Briefly, the rats were placed in flat plate rat fixators made of plexiglass and fixed with gates at the head and tail. The fixator was well ventilated and the space could be adjusted based on the sizes of the rats. Restraint was performed from 9:00 a.m-12:00 p.m. each day, with water and food prohibited during this time period. Rats from the DRP group were additionally treated with a propranolol [propranolol tablets (cat. no. E200704; Jiangsu Yabang Alpusen Pharmaceutical Co., Ltd.)] water suspension at a dose of 10 mg/kg body weight, which was administered via gavage once a day. When rats exhibited no food and/or water intake state, dyspnea and autotomy (self-mutilation), they were euthanized; euthanasia was confirmed by the lack of pupillary reflex to light.

*Tissue sampling*. After 8 weeks, all rats were anesthetized via inhalation of isoflurane (induction, 3%; maintenance, 2%) (39,40) and blood samples (1 ml/rat) were collected from the abdominal aorta following a laparotomy. The rats were then euthanized by administering an overdose of sodium pentobarbital (100 mg/kg body weight, intravenously), followed bycervical dislocation. Euthanasia was confirmed by the lack of pupillary reflex to light. The gastric tissues were extracted from the lesser curvature of the stomach, fixed with 4% paraformaldehyde solution at room temperature for 24 h and embedded in paraffin for hematoxylin and eosin (H&E) staining and TUNEL analysis. The samples were frozen at -80˚C prior to their use in subsequent western blotting and reverse transcription-quantitative PCR (RT-qPCR).

*H&E staining*. H&E staining was performed using a commercial H&E kit (cat. no. G1120; Beijing Solarbio Science & Technology Co., Ltd.) according to the manufacturer's instructions. The paraffin-embedded sections (0.4 $\mu$m thick) were dewaxed and successively hydrated with xylene for 8 min twice, 100% ethyl alcohol for 5 min twice, 95% ethyl alcohol for 5 min, 80% ethyl alcohol for 5 min, 70% ethyl alcohol for 5 min and distilled water for 5 min. The sections were then stained with hematoxylin solution for 3 min, differentiation solution for 30 sec and eosin dye for 1 min. Subsequently, the sections were processed with 95% ethyl alcohol for 5 min twice, 100% ethyl alcohol for 5 min twice, xylene for 5 min twice and neutral gum successively. All aforementioned steps were performed at room temperature. The sections were analyzed using an Olympus light microscope (CX31; Olympus

Corporation) and assessed via reference to the new version of The Sydney System (41).

*ELISA*. Serum was collected from the blood via centrifugation at 2,000 x g at 4˚C for 20 min. The levels of epinephrine and norepinephrine were quantified using commercial kits [rat epinephrine ELISA kit (cat. no. ml003213) and rat norepinephrine ELISA kit (cat. no. ml002873); Shanghai Enzyme-linked Biotechnology Co., Ltd]. Briefly, a total of 50 $\mu$l standard substance at various concentrations (epinephrine, 16, 8, 4, 2, 1 and 0 ng/ml; norepinephrine, 8, 4, 2, 1, 0.5 and 0 ng/ml) and 10 $\mu$l samples (diluted at a ratio of 1:5 using 40 $\mu$l sample diluent) were added to the corresponding wells on the ELISA coated plate, followed by the addition of 100 $\mu$l HRP-conjugate reagent to the wells. Sample diluent was added to blank wells only. After being sealed, the plate was incubated at 37˚C for 60 min. After rinsing five times with washing solution, 50 $\mu$l chromogenic agent A and 50 $\mu$l chromogenic agent B were successively added to each well and incubated at 37˚C for 15 min in the dark. Finally, 50 $\mu$l stop solution was used to terminate the reaction at room temperature for 5-10 min. The absorbance at 450 nm was quantified using a microplate reader (MultiskanGO; Thermo Fisher Scientific, Inc.).

*Western blotting*. Total protein was extracted from the tissue samples using RIPA lysis buffer (cat. no. R0020; Beijing Solarbio Science & Technology Co., Ltd.) supplemented with protease inhibitor. Total protein was quantified using a BCA Protein Assay Kit (cat. no. PC0020; Beijing Solarbio Science & Technology Co., Ltd.). The total protein (150 $\mu$g/lane) was separated via SDS-PAGE on an 8% gel and then transferred onto a PVDF membrane at room temperature (300 mA for 3 h). After blocking with 5% skimmed milk dissolved in TBS containing 0.5% Tween-20 (TBST) at room temperature for 1 h, the membrane was incubated with the following primary antibodies: Akt (rabbit polyclonal antibody; 1:300; cat. no. bs-6951R; BIOSS), phosphorylated (p)-Akt (mouse monoclonal antibody; 1:100; cat. no.sc-514032; Santa Cruz Biotechnology, Inc.), p53 (rabbit polyclonal antibody; 1:500; 10442-1-AP; ProteinTech Group, Inc.), p-p53 (Thr18; rabbit polyclonal antibody; 1:500; cat. no.bs-3710R; BIOSS), ADRB2 (rabbit polyclonal antibody; 1:500; cat. no.bs-0947R; BIOSS) and the loading control GAPDH (rabbit polyclonal antibody; 1:1,000; cat. no.bs-10900R; BIOSS) overnight at 4˚C. After being rinsed with TBST three times for 5 min each, the membrane was then incubated with secondary antibodies against goat anti-rabbit IgG H&L/HRP (1:10,000; cat. no. bs-40295G-HRP; BIOSS) or mouse anti-mouse m-IgGκBP-HRP (1:6,000; cat. no. sc-516102; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature, followed by rinsing three times with TBST for 5 min each. Finally, the membrane was visualized using ECL Ultra-sensitive Luminescent Liquid (cat. no. PE0010, Beijing Solarbio Science & Technology Co., Ltd.) using a chemiluminescence imaging system (FluorChem Q; ProteinSimple). The gray values of the protein bands were analyzed using ImageJ software (version 1.6; National Institutes of Health).

*RT-qPCR*. Total RNA was isolated using TRIzon® reagent from gastric tissues (cat. no.CW0580; CoWin Biosciences) and was quantified using a NanoDrop One$^{C}$ UV-Vis Spectrophotometer (Thermo Fisher Scientific, Inc.). Complementary DNA (cDNA) was synthesized using a HiFiScript cDNA Synthesis Kit (cat. no. CW2569M; CoWin Biosciences) according to the manufacturer's protocol. qPCR was performed using an UltraSYBR Mixture (Low ROX) kit following the manufacturer's instructions (cat. no. CW2601M; CoWin Biosciences) on a 7500 Fast detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.). qPCR was performed in a 20 $\mu$l reaction system consisting of ~50 ng cDNA, 10 $\mu$l UltraSYBR Mixture (Low ROX) and 0.2 $\mu$M forward and reverse primers for each target. qPCR was performed using the following thermocycling conditions: Initial denaturation at 95˚C for 10 min, followed by amplification for 40 cycles (denaturation at 95˚C for 15 sec and annealing and extension at 60˚C for 1 min). The relative mRNA expression levels were analyzed using the $2^{-\Delta\Delta Cq}$ method (42) and were normalized to β-actin. The primers used in the present study are presented in Table I.

Table I. Primers used in the present study.

| Gene | Sequence (5'-3') |
|---|---|
| Akt | F: GAGGTTGCCCACACGCTTA |
| | R: GGTCGTGGGTCTGGAATGAG |
| p53 | F: GAGTTGTTAGAAGGCCCAGAGG |
| | R: AGAAGGGACGGAAGATGACAG |
| β-actin | F: TGTCACCAACTGGGACGATA |
| | R: GGGGTGTTGAAGGTCTCAAA |

F, forward; R, reverse.

*TUNEL assay*. Cell apoptosis was detected using a one-step TUNEL apoptosis assay kit (cat. no. C1088; Beyotime Institute of Biotechnology). The paraffin-embedded tissue sections (0.4 $\mu$m) were dewaxed using xylene and gradient ethanol solutions at room temperature (xylene for 10 min, xylene for 8 min, 100% ethyl alcohol for 5 min, 90% ethyl alcohol for 2 min, 70% ethyl alcohol for 2 min and distilled water for 2 min successively). Samples were incubated with 20 $\mu$g/ml proteinase K (DNase-free) at 37˚C for 15 min. After being washed with PBS three times, the sections were incubated with TUNEL reaction solution at 37˚C for 1 h. The sections were sealed with antifade mounting medium (containing DAPI) prior to another rinse with PBS. The apoptotic ratio was defined as the ratio of apoptotic cells to total cells when viewed under a fluorescence microscope (CX31; Olympus Corporation). A total of five representative fields of view were assessed from each group.

*Statistical analysis*. SPSS software (version 23.0; IBM Corp.) was used for statistical analysis. Data are presented as the means ± standard deviation. The samples for TUNEL analysis, RT-qPCR and western blotting were obtained from 5 rats/group. For H&E staining and ELISA analysis, the samples were obtained from all the rats/each group (no. of rats in each group: 6 for control, 8 for DD, 5 for DR and 7 for DRP). One-way ANOVA followed by Tukey's post hoc test was used

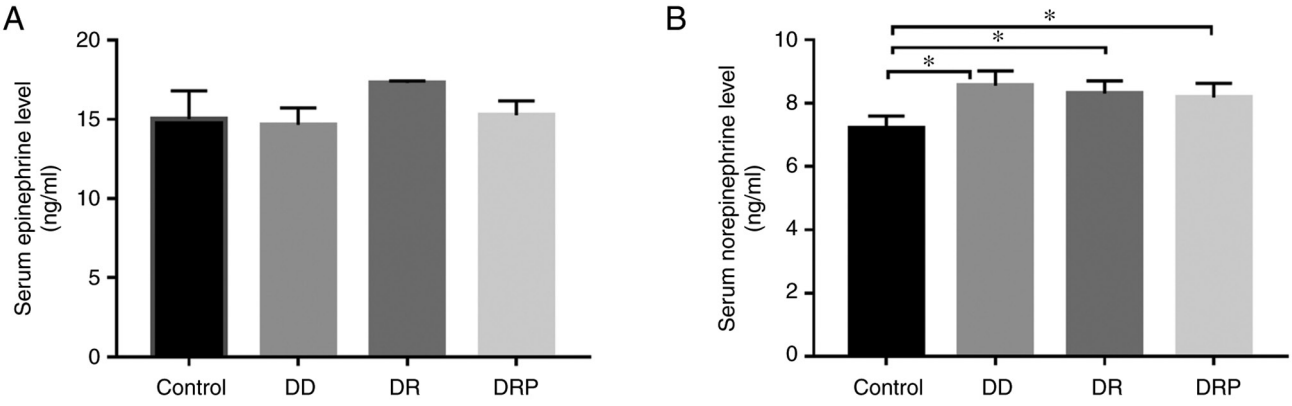


Figure 1. Chronic restraint stress does not significantly affect the serum levels of epinephrine or norepinephrine. (A) ELISA of serum epinephrine levels. (B) ELISA of serum norepinephrine levels. *P<0.05. DD, detrimental diet group; DR, detrimental diet with chronic restraint group; DRP, detrimental diet with chronic restraint and propranolol administration group.

for statistical comparisons between three or more normally distributed and continuous groups. Statistical comparisons of non-normally distributed data and/or data of uneven variance were performed using the Kruskal-Wallis H nonparametric test and the Nemenyi post hoc test. Fisher's exact probability test was applied to analyze pathological data. P<0.05 was considered to indicate a statistically significant difference.

## Results

*Chronic restraint stress does not significantly affect the levels of serum epinephrine or norepinephrine*. No significant difference was observed in the levels of serum epinephrine among all groups (P=0.065; Fig. 1A). Compared with in the control group, the levels of serum norepinephrine were significantly increased in the DD, DR or DRP group (DD vs. control, P=0.011; DR vs. control, P=0.026; DRP vs. control, P=0.033); this result may be due to the same gavage that was administered to all three treatment groups (Fig. 1B). However, the levels of both serum epinephrine and norepinephrine did not differ significantly between the DD, DR and DRP groups. With regard to the levels of serum norepinephrine, there was no significant difference between the DD and DR groups, which indicated that chronic restraint stress had no significant effect on it. These findings indicated that chronic restraint stress did not significantly affect the levels of serum epinephrine or norepinephrine.

*Adrenergic β receptor inhibitor propranolol mitigates stress-induced stomach pathological changes*. The histology data, including inflammation, atrophy, metaplasia, dysplasia and severity are presented in Table II. The results demonstrated that dysplasia was only observed in the DD (2/8 rats; 25%) and DR (4/5 rats; 80%) groups, and the total dysplasia rate of the DR group was significantly higher than that of other three groups (P<0.05). In addition, the total atrophy rate of the DR group was significantly higher than that of the control group (P<0.05); however, regarding the rate of intestinal metaplasia, there was no significant difference between each group. Atrophic changes and dysplasia were markedly reduced by propranolol, an inhibitor of ADRB2 (Fig. 2A). Gross changes to the stomach included a thinner gastric wall and reduced elasticity in the DD, DR and DRP groups compared with in the control group (Fig. 2B). Furthermore, marked pathological changes were potentially induced by the detrimental diet and aggravated by chronic restraint stress, which were markedly reduced by propranolol. These results suggested that chronic restraint stress may exert detrimental effects via ADRB2.

*Chronic restraint stress reduces gastric epithelial apoptosis via the ADRB2 signaling pathway*. Significant differences in apoptotic ratios were observed among the four groups (P=0.002). Compared with the control, the apoptotic ratio of gastric epithelial cells in the DD, DR and DRP groups were significantly decreased (P<0.05), with the DR group exhibiting the lowest apoptotic ratio (P=0.001). Compared with the DD group, the apoptotic ratio was decreased in the DR group (P=0.283), whereas it was elevated in the DRP group (P=0.988); however, these findings were not statistically significant (Fig. 3). This suggested that chronic restraint stress potentially reduces gastric epithelial apoptosis via the ADRB2 signaling pathway.

*Chronic restraint stress aggravates the effects induced by a detrimental diet via the Akt/p53 signaling pathway mediated via ADRB2*. As presented in Fig. 4, the mRNA expression levels of Akt and p53 differed significantly among the four groups (Akt, P=0.000; p53, P=0.000). The DD treatment group exhibited significantly increased Akt mRNA expression levels compared with the control (P=0.007). Moreover, the Akt mRNA expression levels were significantly enhanced in the DR group compared with the DD group (P=0.001). Akt mRNA expression levels were significantly decreased in the DRP group compared with the DR group (P=0.000). The mRNA expression levels of p53 were also significantly upregulated in the DD group compared with the control (P=0.002), whereas they were significantly reduced in the DR and DRP groups compared with in the DD group (DR vs. DD, P=0.002; DRP vs. DD, P=0.001).

The protein expression levels of ADRB2, Akt and p-Akt, along with the ratio of p-p53/p53 differed significantly among the four groups (P=0.000, P=0.018, P=0.009 and P=0.006, respectively). The protein expression levels of ADRB2, Akt and p-Akt were not significantly elevated in the DD group but

Table II. Incidence of lesions in gastric mucosa.

| Group | No. of rats | No. with inflammation (%) | | | No. with atrophy (%)[a] | | | No. with intestinal metaplasia (%)[b] | | | No. with dysplasia (%)[c] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mild | Moderate | Severe | Mild | Moderate | Severe | Mild | Moderate | Severe | Mild | Moderate | Severe |
| Control | 6 | 4 (66.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (16.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| DD | 8 | 0 (0.0) | 5 (62.5) | 3 (37.5) | 0 (0.0) | 2 (25.0) | 2 (25.0) | 2 (25.0) | 2 (25.0) | 1 (12.5) | 0 (0.0) | 0 (0.0) | 2 (25.0) |
| DR | 5 | 1 (20.0) | 0 (0.0) | 4 (80.0) | 2 (40.0) | 1 (20.0) | 1 (20.0) | 0 (0.0) | 1 (20.0) | 1 (20.0) | 0 (0.0) | 1 (20.0) | 3 (60.0) |
| DRP | 7 | 0 (0.0) | 1 (14.3) | 6 (85.7) | 3 (42.9) | 0 (0.0) | 0 (0.0) | 2 (28.6) | 1 (14.3) | 1 (14.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) |

[a]The total atrophy rate of the DR group was significantly different compared with the control group ($P<0.05$). [b]There was no significant difference in the rate of intestinal metaplasia among all groups. [c]The total rate of dysplasia in the DR group was significantly different from that in the other three groups ($P<0.05$). DD, detrimental diet group; DR, detrimental diet with chronic restraint group; DRP, detrimental diet with chronic restraint and propranolol treatment group.

were significantly elevated in the DR group compared with the control. These effects on ADRB2 and p-Akt were significantly reduced via propranolol treatment compared with the DR treatment group (Fig. 5A-D). The ratio of p-Akt/Akt was not significantly different among all groups ($P=0.608$; Fig. 5E), but it did exhibit a similar trend to that of the ADRB2, Akt and p-Akt protein expression levels. The ratio of p-p53/p53 was significantly decreased in the DD and DR groups compared with the control (DD vs. control, $P=0.048$; DR vs. control, $P=0.01$; Fig. 5F). The decreased ratio of p-p53/p53 caused by DR treatment was significantly alleviated by propranolol treatment ($P=0.049$). These data suggested that chronic restraint stress potentially aggravated the negative effects induced by the detrimental diet via the Akt/p53 signaling pathway mediated via ADRB2.

## Discussion

Gastric carcinogenesis is canonically described as a multi-step process driven by numerous etiological factors (43,44). Among these, cadherin 1 mutations cause diffuse types of GC with a poor clinical prognosis in the early stages of life (7,45). However, the majority of patients suffer from another type of intestinal GC, which canonically follows the Correa pattern (46). This therefore indicates that most GC cases are caused by acquired etiological factors, which could possibly be prevented or avoided. However, genetic alterations may also serve a predisposing role (44). Moreover, among these preventable factors, psychosocial stress has recently received increased attention.

The Akt/p53 signaling pathway, which is activated by various types of stressful situations, has been reported to be involved in the development of numerous types of cancer (21,47-49). The Akt/p53 signaling pathway is closely associated with both genetic mutations and an impaired ability to monitor genetic mutations in cells (50-53). The adrenergic signaling pathway, activated under various stressful situations, exerts adverse effects on the immune surveillance ability of cells via various mechanisms, including elevated receptors expression, post-translation modifications and damage to both natural and adaptive immunity (23,28,34,54-59).

The ADRB2 signaling pathway has previously been reported to be activated under numerous stressful situations (23,28,34,36,55). In the present study, no significant changes were observed in the serum levels of epinephrine and norepinephrine in the restraint groups compared with the DD group, which indicated that chronic stress did not activate the adrenergic signaling pathway via endocrine mechanisms. The high expression of ADRB2 protein in the DR group suggested that the ADRB2 signaling pathway may serve an essential role in stress-related chronic inflammation and carcinogenesis in gastric tissues, which is consistent with the conclusions reached in the previous studies published by Zhang *et al* (23) and Shi *et al* (36).

The detrimental diet, which contained MNNG, resulted in the significant inhibition of gastric epithelial apoptosis. A previous study by Ohyama *et al* (60) reported that the effects of MNNG on apoptosis were time-sensitive and that short-term exposure to MNNG induced apoptosis, with the effects decreasing over time. Moreover, Cai *et al* (61) reported the inhibitory effect of long-term exposure to MNNG on

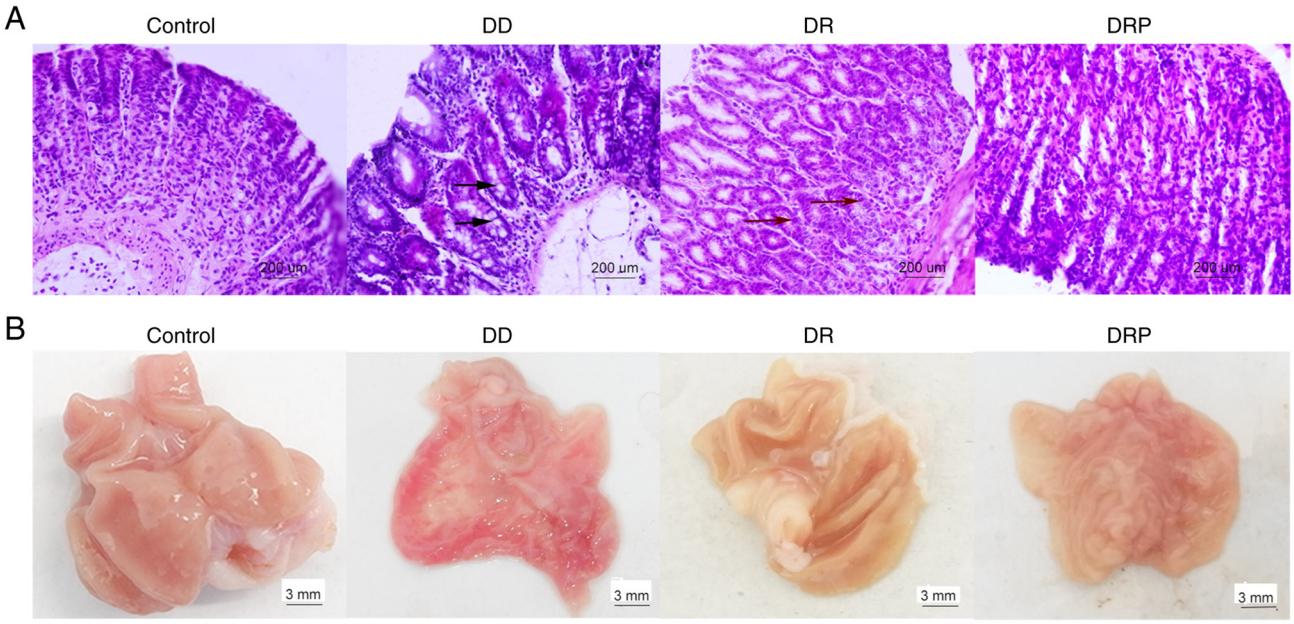


Figure 2. Propranolol mitigates stress-induced stomach pathological changes. (A) Histological changes in gastric tissues were determined via H&E staining (magnification, x400; scale bar, 200 $\mu$m). A large number of goblet cells (black arrow) in the DD group and atypical cells (with numerous mitotic phases; indicated by the brown arrow) in the DR group were observed. (B) Gross changes to the stomach (scale bar, 3 mm). DD, detrimental diet group; DR, detrimental diet with chronic restraint group; DRP, detrimental diet with chronic restraint and propranolol treatment group.

apoptosis; this previous study showed that MNNG exposure enhanced the role of anti-apoptotic proteins (Bcl-2 and Bcl-XL) and attenuated the expression of a pro-apoptotic protein (caspase-3). In the present study, long-term exposure to high-concentrations of MNNG for 8 weeks exerted similar inhibitory effects on the apoptosis of gastric epithelial cells. Furthermore, the present study demonstrated that chronic stress may further reduce gastric epithelial cell apoptosis via the ADRB2 signaling pathway. The apoptotic ratio of the gastric epithelium was 23% in the control group, which was slightly higher than the ratio (15-20%) reported in previous studies (62-64). The lowest apoptotic ratio was observed in the DR group (10.9%) and was markedly alleviated in the DRP group (14.8%), which suggested that the ADRB2 signaling pathway potentially served a crucial role in stress-related apoptosis.

To explore the underlying mechanisms of stress-related apoptosis, the present study analyzed the expression levels of Akt and p53. Gene expression may be regulated at various levels, such as transcription, translation and post-translation modification (65,66). In the present study, the detrimental diet mainly influenced the mRNA expression levels of Akt and p53 due to the genotoxic effects of MNNG, in accordance with the data from a previous study published by Fang *et al* (67). Chronic restraint stress significantly enhanced the mRNA and protein expression levels of Akt induced by the detrimental diet, which was markedly reversed via propranolol. Furthermore, by considering the similar changes in the Akt phosphorylation level, it can be hypothesized that Akt may serve as a pivotal downstream molecule of the ADRB2 signaling pathway. However, the activation of Akt via chronic restraint stress may promote the ubiquitination and degradation of active p-p53 via phosphorylation of Akt's downstream molecule MDM2, which thereby reduces the effects of p53 (21,68).

In the present study, the combined detrimental diet and chronic restraint stress rat model demonstrated that the Akt/p53 signaling pathway functioned downstream of ADRB2 (Fig. 6). Expression levels of Akt may serve a key role, in consideration of variations in its expression in the DR and DRP groups similar to the variations of ADRB2 protein expression levels induced by chronic restraint stress and propranolol. The ubiquitinationof p53 has been reported to be significantly enhanced by the activation of Akt (69) via the ADRB2 signaling pathway, through which the p53 efficiency and fate of epithelial cells are finely regulated (70,71). p53 also serves as a pivotal molecule in the interaction of cellular survival, apoptosis and proliferation (72). It has been previously demonstrated that the stress-related ADRB2 signaling pathway may not affect the mRNA expression levels of p53, but the protein expression levels of p53 (73,74).

There were several limitations to the present study. The limited number of animal samples and the imbalance of group sizes weakened the reliability of the results of the study. The unstressed groups should have included a control group with a standard diet and a sham group with a detrimental diet, sham group with a standard diet with propranolol and a sham group with detrimental diet with propranolol. The stressed groups should have included stress groups with a standard diet, a detrimental diet, a standard diet with propranolol and a detrimental diet with propranolol. The absence of sham groups made it more difficult to evaluate the roles of each factor employed and led to the conclusions of the present study being less comprehensive. The statistically significant results among the three non-control groups (which were administered the same gavage) indicated that the results obtained under the current grouping situation were still of clinical significance to a certain extent. Furthermore, each treatment group included certain samples with unclear or missing blots in the

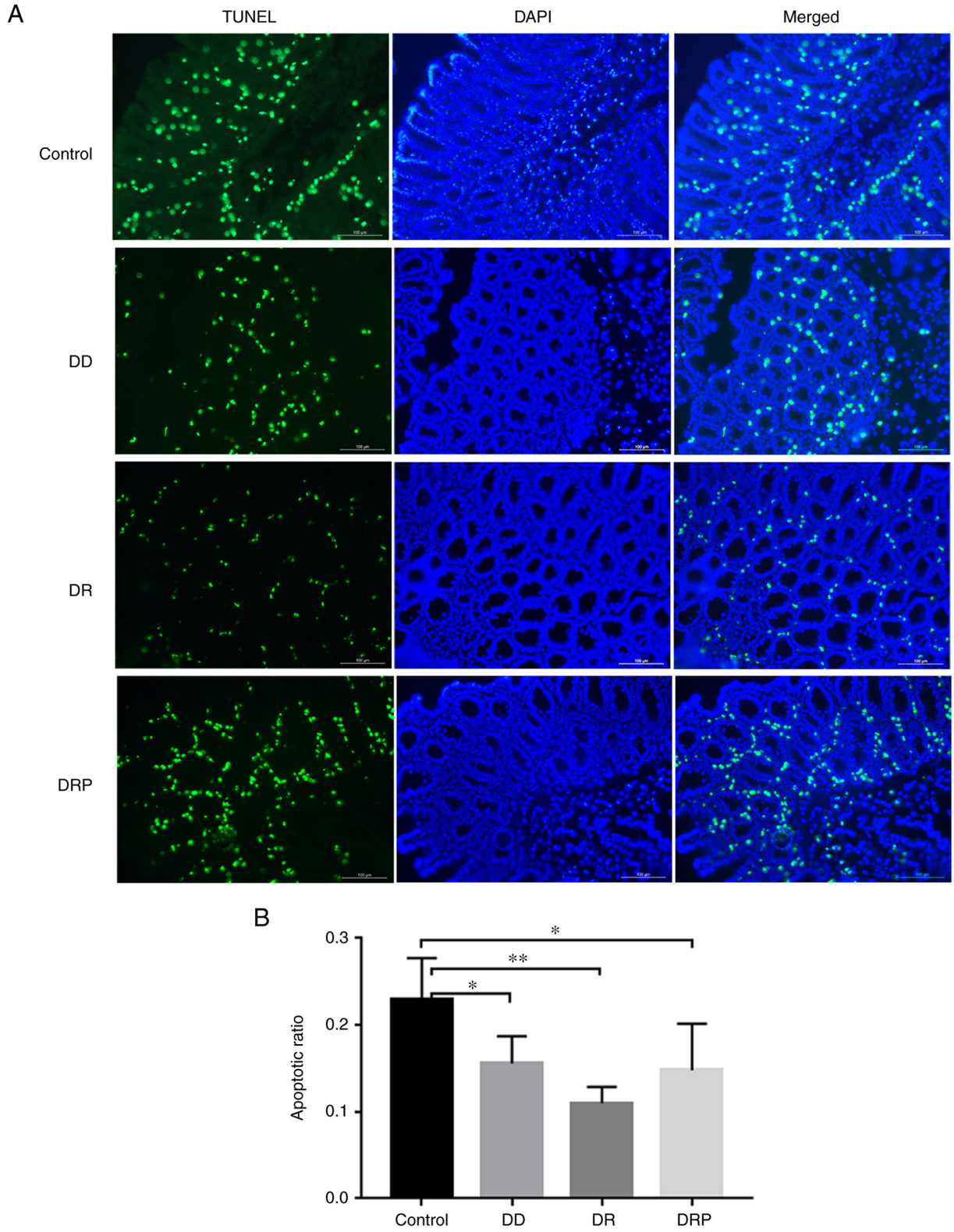


Figure 3. Chronic restraint stress reduces gastric epithelial apoptosis via the adrenoceptor β2 signaling pathway. Apoptosis of gastric epithelial cells in each of the treatment groups was analyzed using the TUNEL assay (TUNEL, DAPI and merged figures were derived from the same area). (A) TUNEL staining of gastric epithelial cells in each group. Magnification, x200; scale bar, 100 $\mu$m. (B) Apoptotic ratio of gastric epithelial cells in each group. $^{*}$P<0.05 and $^{**}$P<0.01. DD, detrimental diet group; DR, detrimental diet with chronic restraint group; DRP, detrimental diet with chronic restraint and propranolol treatment group.

western blotting analyses. Different rats may have responded differently to the same pathological conditions, leading to differential modeling results, whereas re-probing with different antibodies on the stripped membrane and antibodies from different manufacturers may have affected the band densities. For example, the p-p53 levels in certain samples are higher than those of p53. As the molecular weight of p53 and ADRB2 is very similar (53 and 46 kDa, respectively), the

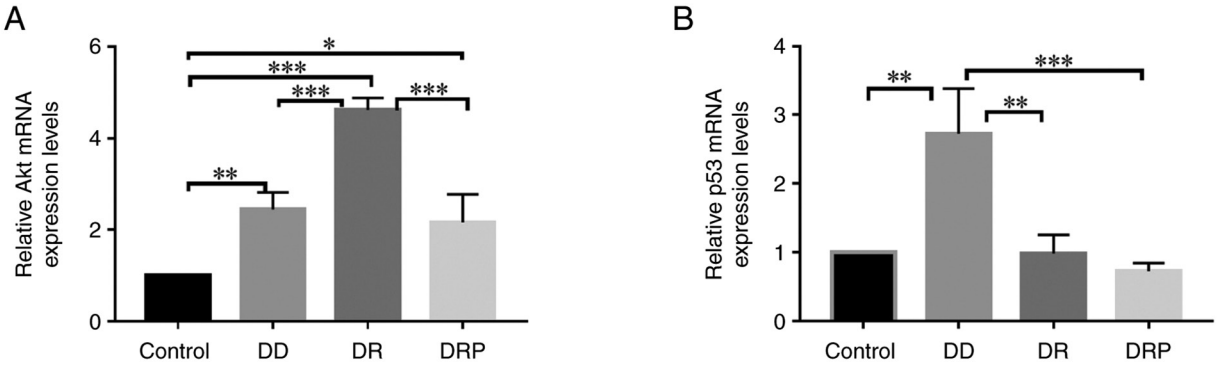


Figure 4. Detrimental diet upregulates the mRNA expression levels of Akt and p53, whereas restraint stress only enhances the mRNA expression levels of Akt. mRNA expression levels of (A) Akt and (B) p53 were determined using reverse transcription-quantitative PCR. $^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001. DD, detrimental diet group; DR, detrimental diet with chronic restraint group; DRP, detrimental diet with chronic restraint and propranolol treatment group.

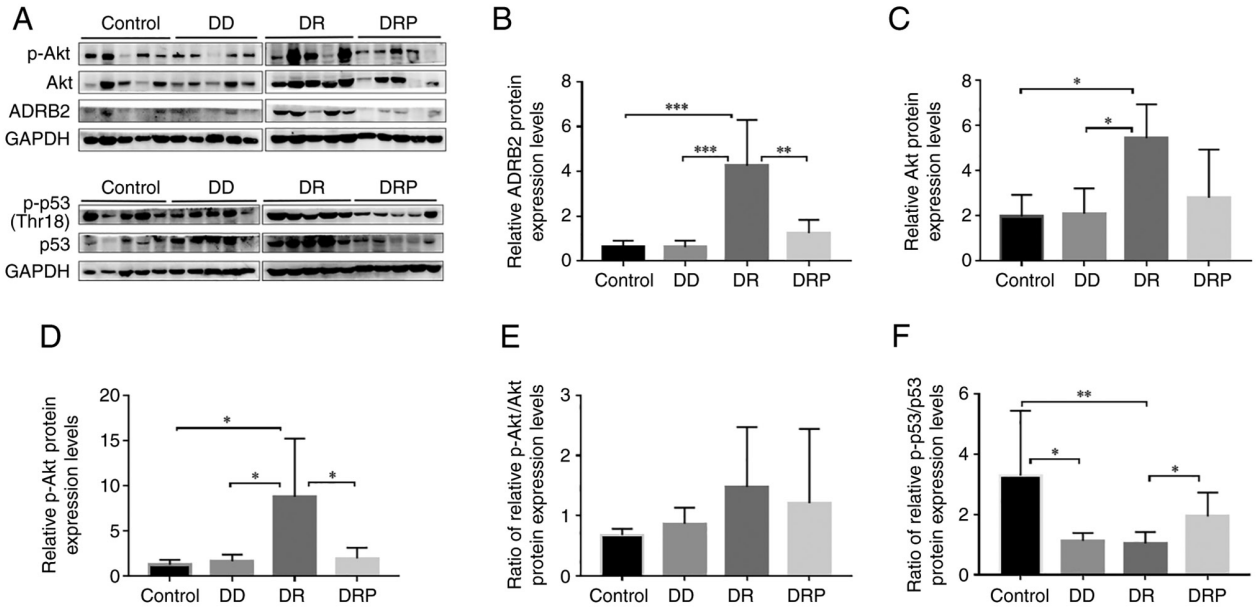


Figure 5. Chronic restraint stress increases the effects induced by the detrimental diet via the Akt/p53 signaling pathway mediated via ADRB2. Protein expression levels in gastric tissues were detected via western blotting. (A) Representative western blotting bands and the semi-quantification of (B) ADRB2; (C) Akt; (D) p-Akt; (E) p-Akt/Akt; and (F) p-p53/p53. $^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001. ADRB2, adrenoceptor β2; p, phosphorylated; DD, detrimental diet group; DR, detrimental diet with chronic restraint group; DRP, detrimental diet with chronic restraint and propranolol treatment group.

band of ADRB2 may have been partially disrupted during the membrane segmentation (75-78). The appearance of uneven loading of the blots for GAPDH may be due to variation in the protein expression levels of housekeeping genes under certain conditions such as hypoxia and exercise. Moreover, high levels of post-translation modification in housekeeping genes may have exerted certain adverse effects on their quantification, which depends on the epitope of the antibodies employed (79,80).

Stress-related adrenergic signaling functions in a complex manner and additional external factors, other than those considered in the present study, may also exert effects on it at specific levels, such as during translation and post-translation modification. Furthermore, other G-protein-coupled receptors, such as ADRB3, and downstream signaling pathways, such as MAPKs, may be involved in chronic restraint-stress induced carcinogenesis (81-83). The status of MDM2, as an important effector of the Akt/p53 signaling pathway, should have also been considered. Therefore, further experiments are required to bridge the gap between chronic restraint stress, the ADRB2 signaling pathway and apoptosis.

In conclusion, the present study demonstrated that chronic restraint stress increased gastric epithelial malignant transformation induced by a detrimental diet, at least partially via the Akt/p53 signaling pathway mediated via ADRB2. These results suggested that gastric carcinogenesis is an integral process driven via complex etiological factors. Therefore, holistic intervention may be necessary, including more dedicated psychosocial care, alongside precise individual clinical treatments.

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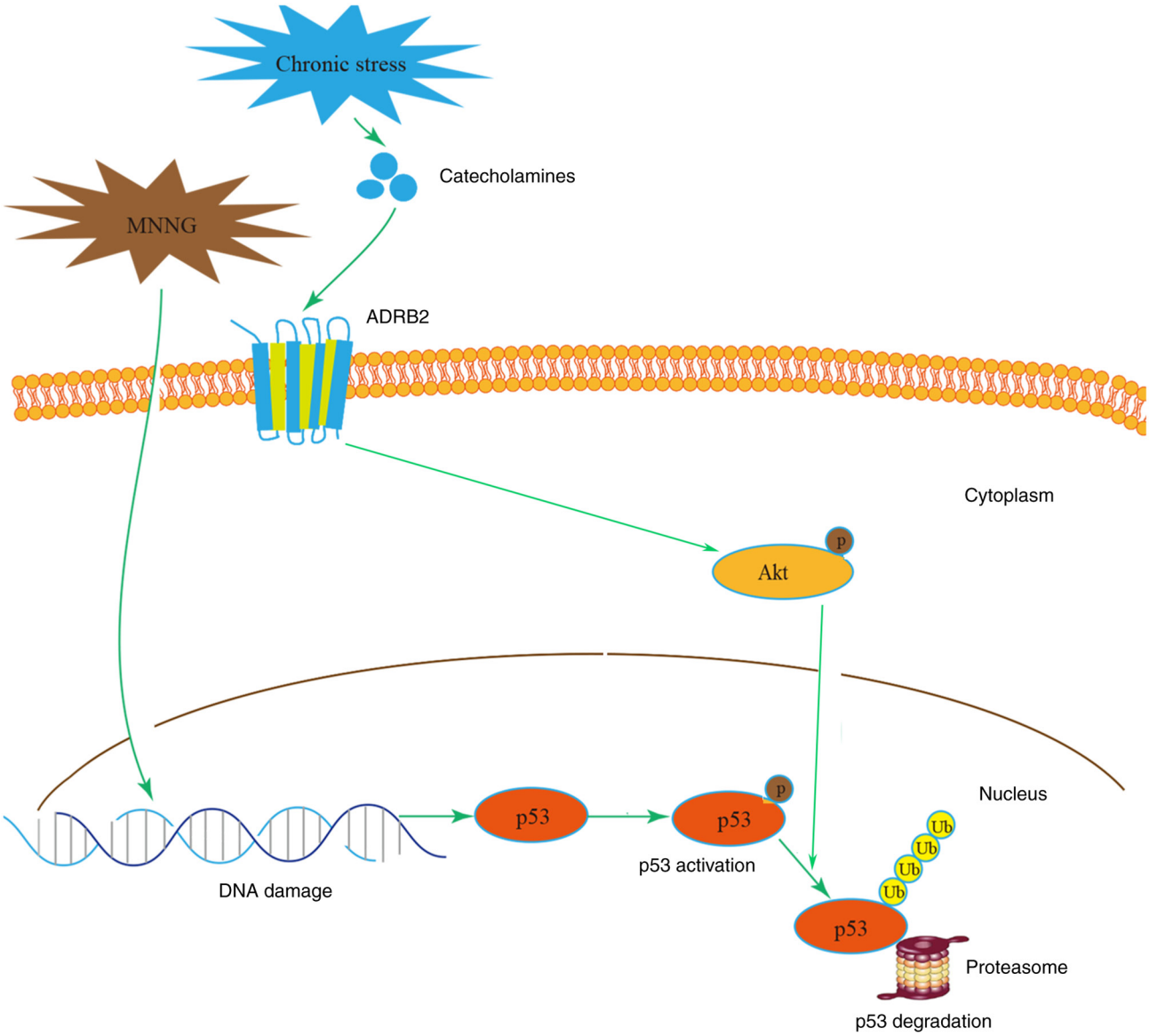


Figure 6. Putative mechanism via which chronic restraint stress affects gastric epithelial transformation. A detrimental diet resulted in DNA damage and activated the protein expression of p53. Restraint stress may act on ADRB2 receptors by promoting catecholamine released from the local nerve terminals of the gut, thus inducing the activation of Akt, which may contribute to the ubiquitination and degradation of p53 and thereby reduce the effects of p53. The apoptosis of epithelial cells was finely regulated by p53. ADRB2, adrenoceptor β2; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; p, phosphorylated.

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**Availability of data and materials**

The data used to support the findings of this study are available from the corresponding author on reasonable request.

**Authors' contributions**

WJ and CZ designed the study. CZ, MY and XG performed the experiments. CZ analyzed the data and drafted the initial manuscript. WJ supervised the present study and revised the manuscript. WJ and CZ confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

**Ethics approval and consent to participate**

All experiments were approved by the Experimental Ethics Committee of Weifang Medical University (Weifang, China; approval no. 2020SDL166).

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

## References


1. Thrift AP and El-Serag HB: Burden of gastric cancer. Clin Gastroenterol Hepatol 18: 534-542, 2020.
2. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A and Bray F: Cancer statistics for the year 2020: An overview. Int J Cancer: Apr 5, 2021 doi: 10.1002/ijc.33588 (Epub ahead of print).
3. Waldum HL, Kleveland PM and Sørdal ØF: *Helicobacter pylori* and gastric acid: An intimate and reciprocal relationship. Therap Adv Gastroenterol 9: 836-844, 2016.
4. Loogna P, Franzén L, Sipponen P and Domellöf L: *Helicobacter pylori*, N-methyl-N'-nitro-N'-nitrosoguanidine, and bile modulate gastric cell kinetics in experimental cancer. Virchows Arch 439: 653-660, 2001.
5. Bagheri V, Memar B, Momtazi AA, Sahebkar A, Gholamin M and Abbaszadegan MR: Cytokine networks and their association with *Helicobacter pylori* infection in gastric carcinoma. J Cell Physiol 233: 2791-2803, 2018.
6. Bekaii-Saab T and El-Rayes B: Identifying and targeting cancer stem cells in the treatment of gastric cancer. Cancer 123: 1303-1312, 2017.
7. Luo W, Fedda F, Lynch P and Tan D: CDH1 gene and hereditary diffuse gastric cancer syndrome: Molecular and histological alterations and implications for diagnosis and treatment. Front Pharmacol 9: 1421, 2018.
8. Oliveira C, Pinheiro H, Figueiredo J, Seruca R and Carneiro F: Familial gastric cancer: Genetic susceptibility, pathology, and implications for management. Lancet Oncol 16: e60-e70, 2015.
9. Ohnishi S, Ma N, Thanan R, Pinlaor S, Hammam O, Murata M and Kawanishi S: DNA damage in inflammation-related carcinogenesis and cancer stem cells. Oxid Med Cell Longev 2013: 387014, 2013.
10. Costa L, Corre S, Michel V, Le Luel K, Fernandes J, Ziveri J, Jouvion G, Danckaert A, Mouchet N, Da Silva Barreira D, *et al*: USF1 defect drives p53 degradation during *Helicobacter pylori* infection and accelerates gastric carcinogenesis. Gut 69: 1582-1591, 2020.
11. Ushijima T, Nakajima T and Maekita T: DNA methylation as a marker for the past and future. J Gastroenterol 41: 401-407, 2006.
12. Toyoda T, Tsukamoto T, Yamamoto M, Ban H, Saito N, Takasu S, Shi L, Saito A, Ito S, Yamamura Y, *et al*: Gene expression analysis of a *Helicobacter pylori*-infected and high-salt diet-treated mouse gastric tumor model: Identification of CD177 as a novel prognostic factor in patients with gastric cancer. BMC Gastroenterol 13: 122, 2013.
13. Moreno-Villanueva M and Bürkle A: Stress hormone-mediated DNA damage response-implications for cellular senescence and tumour progression. Curr Drug Targets 17: 398-404, 2016.
14. Oren M: Decision making by p53: Life, death and cancer. Cell Death Differ 10: 431-442, 2003.
15. Hu W, Feng Z and Levine AJ: The regulation of multiple p53 stress responses is mediated through MDM2. Genes Cancer 3: 199-208, 2012.
16. Abraham AG and O'Neill E: PI3K/Akt-mediated regulation of p53 in cancer. Biochem Soc Trans 42: 798-803, 2014.
17. Bálint EE and Vousden KH: Activation and activities of the p53 tumour suppressor protein. Br J Cancer 85: 1813-1823, 2001.
18. Tan BX, Liew HP, Chua JS, Ghadessy FJ, Tan YS, Lane DP and Coffill CR: Anatomy of Mdm2 and Mdm4 in evolution. J Mol Cell Biol 9: 3-15, 2017.
19. Wang Y, Sun H, Xiao Z, Zhang G, Zhang D, Bao X, Li F, Wu S, Gao Y and Wei N: DNA damage and apoptosis induced by a potent orally podophyllotoxin derivative in breast cancer. Cell Commun Signal 16: 52, 2018.
20. Zhu Y, Dai B, Zhang H, Shi G, Shen Y and Ye D: Long non-coding RNA LOC572558 inhibits bladder cancer cell proliferation and tumor growth by regulating the AKT-MDM2-p53 signaling axis. Cancer Lett 380: 369-374, 2016.
21. Chibaya L, Karim B, Zhang H and Jones SN: Mdm2 phosphorylation by Akt regulates the p53 response to oxidative stress to promote cell proliferation and tumorigenesis. Proc Natl Acad Sci USA 118: e2003193118, 2021.
22. Huang H, Park S, Zhang H, Park S, Kwon W, Kim E, Zhang X, Jang S, Yoon D, Choi SK, *et al*: Targeting AKT with costunolide suppresses the growth of colorectal cancer cells and induces apoptosis in vitro and in vivo. J Exp Clin Cancer Res 40: 114, 2021.
23. Zhang X, Zhang Y, He Z, Yin K, Li B, Zhang L and Xu Z: Chronic stress promotes gastric cancer progression and metastasis: An essential role for ADRB2. Cell Death Dis 10: 788, 2019.
24. Molina-Castro S, Pereira-Marques J, Figueiredo C, Machado JC and Varon C: Gastric cancer: Basic aspects. Helicobacter: 22 (Suppl 1), 2017 doi: 10.1111/hel.12412.
25. Qiao G, Chen M, Bucsek MJ, Repasky EA and Hylander BL: Adrenergic signaling: A targetable checkpoint limiting development of the antitumor immune response. Front Immunol 9: 164, 2018.
26. Wohleb ES, Hanke ML, Corona AW, Powell ND, Stiner LM, Bailey MT, Nelson RJ, Godbout JP and Sheridan JF: β-Adrenergic receptor antagonism prevents anxiety-like behavior and microglial reactivity induced by repeated social defeat. J Neurosci 31: 6277-6288, 2011.
27. Sgambato D, Capuano A, Sullo MG, Miranda A, Federico A and Romano M: Gut-brain axis in gastric mucosal damage and protection. Curr Neuropharmacol 14: 959-966, 2016.
28. Zhi X, Li B, Li Z, Zhang J, Yu J, Zhang L and Xu Z: Adrenergic modulation of AMPK-dependent autophagy by chronic stress enhances cell proliferation and survival in gastric cancer. Int J Oncol 54: 1625-1638, 2019.
29. Feng Z, Liu L, Zhang C, Zheng T, Wang J, Lin M, Zhao Y, Wang X, Levine AJ and Hu W Chronic restraint stress attenuates p53 function and promotes tumorigenesis. Proc Natl Acad Sci USA 109: 7013-7018, 2012.
30. Tang J, Li Z, Lu L and Cho CH: β-Adrenergic system, a backstage manipulator regulating tumour progression and drug target in cancer therapy. Semin Cancer Biol 23: 533-542, 2013.
31. Kim-Fuchs C, Le CP, Pimentel MA, Shackleford D, Ferrari D, Angst E, Hollande F and Sloan EK: Chronic stress accelerates pancreatic cancer growth and invasion: A critical role for beta-adrenergic signaling in the pancreatic microenvironment. Brain Behav Immun 40: 40-47, 2014.
32. Le CP, Nowell CJ, Kim-Fuchs C, Botteri E, Hiller JG, Ismail H, Pimentel MA, Chai MG, Karnezis T, Rotmensz N, *et al*: Chronic stress in mice remodels lymph vasculature to promote tumour cell dissemination. Nat Commun 7: 10634, 2016.
33. Partecke LI, Speerforck S, Käding A, Seubert F, Kühn S, Lorenz E, Schwandke S, Sendler M, Keßler W, Trung DN, *et al*: Chronic stress increases experimental pancreatic cancer growth, reduces survival and can be antagonised by beta-adrenergic receptor blockade. Pancreatology 16: 423-433, 2016.
34. Thaker PH, Han LY, Kamat AA, Arevalo JM, Takahashi R, Lu C, Jennings NB, Armaiz-Pena G, Bankson JA, Ravoori M, *et al*: Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. Nat Med 12: 939-944, 2006.
35. Lu YJ, Geng ZJ, Sun XY, Li YH, Fu XB, Zhao XY and Wei B: Isoprenaline induces epithelial-mesenchymal transition in gastric cancer cells. Mol Cell Biochem 408: 1-13, 2015.
36. Shi M, Liu D, Duan H, Han C, Wei B, Qian L, Chen C, Guo L, Hu M, Yu M, *et al*: Catecholamine up-regulates MMP-7 expression by activating AP-1 and STAT3 in gastric cancer. Mol Cancer 9: 269, 2010.
37. Liang S, Wang T, Hu X, Luo J, Li W, Wu X, Duan Y and Jin F: Administration of Lactobacillus helveticus NS8 improves behavioral, cognitive, and biochemical aberrations caused by chronic restraint stress. Neuroscience 310: 561-577, 2015.
38. Naert G, Ixart G, Maurice T, Tapia-Arancibia L and Givalois L: Brain-derived neurotrophic factor and hypothalamic-pituitary-adrenal axis adaptation processes in a depressive-like state induced by chronic restraint stress. Mol Cell Neurosci 46: 55-66, 2011.
39. Huang T, Xiao Y, Zhang Y, Wang C, Chen X, Li Y, Ge Y and Gao J: miR-223 ameliorates thalamus hemorrhage-induced central poststroke pain via targeting NLRP3 in a mouse model. Exp Ther Med 23: 353, 2022.
40. Murata I, Sugai T, Murakawa Y, Miyamoto Y, Kobayashi J, Inoue Y and Kanamoto I: Salvianolic acid B improves the survival rate, acute kidney dysfunction, inflammation and NETosis-mediated antibacterial action in a crush syndrome rat model. Exp Ther Med 23: 320, 2022.
41. Dixon MF, Genta RM, Yardley JH and Correa P: Classification and grading of gastritis. The updated Sydney system. International workshop on the histopathology of gastritis, Houston 1994. Am J Surg Pathol 20: 1161-1181, 1996.
42. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
43. Gunathilake M, Lee J, Choi IJ, Kim YI and Kim J: Association between bacteria other than *Helicobacter pylori* and the risk of gastric cancer. Helicobacter 26: e12836, 2021.
44. Machlowska J, Baj J, Sitarz M, Maciejewski R and Sitarz R: Gastric cancer: Epidemiology, risk factors, classification, genomic characteristics and treatment strategies. Int J Mol Sci 21: 4012, 2020.

45. Blair VR, McLeod M, Carneiro F, Coit DG, D'Addario JL, van Dieren JM, Harris KL, Hoogerbrugge N, Oliveira C, van der Post RS, *et al*: Hereditary diffuse gastric cancer: Updated clinical practice guidelines. Lancet Oncol 21: e386-e397, 2020.
46. Kuligowski J, Sanjuan-Herraez D, Vázquez-Sánchez MA, Brunet-Vega A, Pericay C, Ramírez-Lázaro MJ, Lario S, Gombau L, Junquera F, Calvet X and Quintás G: Metabolomic analysis of gastric cancer progression within the Correa's cascade using ultraperformance liquid chromatography-mass spectrometry. J Proteome Res 15: 2729-2738, 2016.
47. Han F, Li CF, Cai Z, Zhang X, Jin G, Zhang WN, Xu C, Wang CY, Morrow J, Zhang S, *et al*: The critical role of AMPK in driving Akt activation under stress, tumorigenesis and drug resistance. Nat Commun 9: 4728, 2018.
48. Sun X, Chen L and He Z: PI3K/Akt-Nrf2 and Anti-inflammation effect of macrolides in chronic obstructive pulmonary disease. Curr Drug Metabolism 20: 301-304, 2019.
49. Zhao Y, Hu X, Liu Y, Dong S, Wen Z, He W, Zhang S, Huang Q and Shi M: ROS signaling under metabolic stress: Cross-talk between AMPK and AKT pathway. Mol Cancer 16: 79, 2017.
50. Kastenhuber ER and Lowe SW: Putting p53 in context. Cell 170: 1062-1078, 2017.
51. Lee JT and Gu W: The multiple levels of regulation by p53 ubiquitination. Cell Death Differ 17: 86-92, 2010.
52. Liu Y, Deisenroth C and Zhang Y: RP-MDM2-p53 pathway: Linking ribosomal biogenesis and tumor surveillance. Trends Cancer 2: 191-204, 2016.
53. Manning BD and Toker A: AKT/PKB signaling: Navigating the network. Cell 169: 381-405, 2017.
54. Agac D, Estrada LD, Maples R, Hooper LV and Farrar JD: The beta2-adrenergic receptor controls inflammation by driving rapid IL-10 secretion. Brain BehavImmun 74: 176-185, 2018.
55. Choy C, Raytis JL, Smith DD, Duenas M, Neman J, Jandial R and Lew MW: Inhibition of β2-adrenergic receptor reduces triple-negative breast cancer brain metastases: The potential benefit of perioperative beta-blockade. Oncol Rep 35: 3135-3142, 2016.
56. Matheis F, Muller PA, Graves CL, Gabanyi I, Kerner ZJ, Costa-Borges D, Ahrends T, Rosenstiel P and Mucida D: Adrenergic signaling in muscularis macrophages limits infection-induced neuronal loss. Cell 180: 64-78.e16, 2020.
57. Mohammadpour H, MacDonald CR, Qiao G, Chen M, Dong B, Hylander BL, McCarthy PL, Abrams SI and Repasky EA: β2 adrenergic receptor-mediated signaling regulates the immunosuppressive potential of myeloid-derived suppressor cells. J Clin Invest 129: 5537-5552, 2019.
58. Nakai A and Suzuki K: Adrenergic control of lymphocyte trafficking and adaptive immune responses. Neurochem Int 130: 104320, 2019.
59. Wirth K and Scheibenbogen C: A unifying hypothesis of the pathophysiology of myalgic Encephalomyelitis/Chronic fatigue syndrome (ME/CFS): Recognitions from the finding of autoantibodies against ss2-adrenergic receptors. Autoimmun Rev 19: 102527, 2020.
60. Ohyama W, Okada E, Fujiishi Y, Narumi K and Yasutake N: In vivo rat glandular stomach and colon micronucleus tests: Kinetics of micronucleated cells, apoptosis, and cell proliferation in the target tissues after a single oral administration of stomach- or colon-carcinogens. Mutat Res 755: 141-147, 2013.
61. Cai T, Zhang C, Zhao Z, Li S, Cai H, Chen X, Cai D, Liu W, Yan Y, Xie K, *et al*: The gastric mucosal protective effects of Astragaloside IV in mnng-induced GPL rats. Biomed Pharmacother 104: 291-299, 2018.
62. Crabtree JE, Jeremy AH, Duval C, Dixon MF, Danjo K, Carr IM, Pritchard DM and Robinson PA: Effects of EGFR inhibitor on *Helicobacter pylori* induced gastric epithelial pathology in vivo. Pathogens 2: 571-590, 2013.
63. Kidd M, Tang LH, Modlin IM, Zhang T, Chin K, Holt PR and Moss SF: Gastrin-mediated alterations in gastric epithelial apoptosis and proliferation in a mastomys rodent model of gastric neoplasia. Digestion 62: 143-151, 2000.
64. Scotiniotis IA, Rokkas T, Furth EE, Rigas B and Shiff SJ: Altered gastric epithelial cell kinetics in *Helicobacter pylori*-associated intestinal metaplasia: Implications for gastric carcinogenesis. Int J Cancer 85: 192-200, 2000.
65. Lipshitz HD, Claycomb JM and Smibert CA: Post-transcriptional regulation of gene expression. Methods 126: 1-2, 2017.
66. Pu M, Chen J, Tao Z, Miao L, Qi X, Wang Y and Ren J: Regulatory network of miRNA on its target: Coordination between transcriptional and post-transcriptional regulation of gene expression. Cell Mol Life Sci 76: 441-451, 2019.
67. Fang MZ, Mar W and Cho MH: Cadmium affects genes involved in growth regulation during two-stage transformation of Balb/3T3 cells. Toxicology 177: 253-265, 2002.
68. Cross B, Chen L, Cheng Q, Li B, Yuan ZM and Chen J: Inhibition of p53 DNA binding function by the MDM2 protein acidic domain. J Biol Chem 286: 16018-16029, 2011.
69. Li J and Kurokawa M: Regulation of MDM2 stability after DNA damage. J Cell Physiol 230: 2318-2327, 2015.
70. Hara MR, Kovacs JJ, Whalen EJ, Rajagopal S, Strachan RT, Grant W, Towers AJ, Williams B, Lam CM, Xiao K, *et al*: A stress response pathway regulates DNA damage through beta2-adrenoreceptors and β-arrestin-1. Nature 477: 349-353, 2011.
71. Hara MR, Sachs BD, Caron MG and Lefkowitz RJ: Pharmacological blockade of a β(2)AR-β-arrestin-1 signaling cascade prevents the accumulation of DNA damage in a behavioral stress model. Cell Cycle 12: 219-224, 2013.
72. Meek DW: Regulation of the p53 response and its relationship to cancer. Biochem J 469: 325-346, 2015.
73. Chen H, Zhang W, Cheng X, Guo L, Xie S, Ma Y, Guo N and Shi M: β2-AR activation induces chemoresistance by modulating p53 acetylation through upregulating Sirt1 in cervical cancer cells. Cancer Sci 108: 1310-1317, 2017.
74. Yang G, Zhang G, Pittelkow MR, Ramoni M and Tsao H: Expression profiling of UVB response in melanocytes identifies a set of p53-target genes. J Invest Dermatol 126: 2490-2506, 2006.
75. Berkelman T: Fluorescent western blotting: High sensitivity detection of multiple targets. Curr Protoc Pharmacol 88: e72, 2020.
76. Gopal A and Herr AE: Multiplexed in-gel microfluidic immunoassays: Characterizing protein target loss during reprobing of benzophenone-modified hydrogels. Sci Rep 9: 15389, 2019.
77. Litovchick L: Stripping of the immunoblot for reprobing. Cold Spring Harb Protoc 2020: 098491, 2020.
78. Sennepin AD, Charpentier S, Normand T, Sarre C, Legrand A and Mollet LM: Multiple reprobing of Western blots after inactivation of peroxidase activity by its substrate, hydrogen peroxide. Anal Biochem 393: 129-131, 2009.
79. Ferguson RE, Carroll HP, Harris A, Maher ER, Selby PJ and Banks RE: Housekeeping proteins: A preliminary study illustrating some limitations as useful references in protein expression studies. Proteomics 5: 566-571, 2005.
80. Ruan W and Lai M: Actin, a reliable marker of internal control? Clin Chim Acta 385: 1-5, 2007.
81. Hotamisligil GS and Davis RJ: Cell signaling and stress responses. Cold Spring Harb Perspect Biol 8: a006072, 2016.
82. Wang P, Hao X, Li X, Yan Y, Tian W, Xiao L, Wang Z and Dong J: Curcumin inhibits adverse psychological stress-induced proliferation and invasion of glioma cells via down-regulating the ERK/MAPK pathway. J Cell Mol Med 25: 7190-7203, 2021.
83. Yong HY, Koh MS and Moon A: The p38 MAPK inhibitors for the treatment of inflammatory diseases and cancer. Expert Opin Investig Drugs 18: 1893-1905, 2009.