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***Basic Study***

**Expression of p-STAT3 and vascular endothelial growth factor in MNNG-induced precancerous lesions and gastric tumor in rats**

Wang XY *et al*. P-STAT3 and VEGF in expression of p-STAT3 and VEGF

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**Abstract**

**AIM**: To investigate the dynamic expression of p-signal transducer and activator of transcription 3 (STAT3) and vascular endothelial growth factor (VEGF) in formation of gastric tumor induced by drinking water with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in Wistar rat.

**METHODS**: One hundred and twenty Wistar rats were randomly divided into two groups (each of 60): Control group and Model group. The sixty rats in each group were randowly divided into three groups (each of 20): C/M15, C/M25 and C/M40 (15, 25 and 40 digital represent the number of feeding weeks from termination). Rats in control group drank normal drinking water freely, rats in model group drank drinking water with 100 ug/mL MNNG freely. Stomach tissues were collected respectively in the end of 15th, 25th and 40th week for microscopic measurement using hematoxylin and eosin staining. The expression of p-STAT3 and VEGF in different pathological types of gastric tissue, such as normal, inflammation, atrophy, hyperplasia and gastric stromal tumor, was observed by immunohistochemistry and Western blot, and analyzed the corelation between the p-STAT3 and VEGF.

**RESULTS:** (1) The expression of p-STAT3 in stomach tissue with gastritis, atrophy, dysplasia and gastric stromal tumor were significantly increased in model group compared with control group (2.5 ± 1.0, 2.75 ± 0.36, 6.2 ± 0.45, 5.67 ± 0.55 *vs* 0.75 ± 0.36, *P* = 0.026, 0.035, 0.001, 0.002, respectively); The expression of p-STAT3 in gastric tissue with dysplasia was higher than its expression in sample with gastritis or atrophy (6.2 ± 0.45 *vs* 2.5 ± 1.0,*P* = 0.006; 6.2 ± 0.45 *vs* 2.75 ± 0.36, *P* = 0.005, respectively); but the expression of p-STAT3 between gastritis and atrophy has no significant difference (*P* > 0.05); (2) the expression of VEGF in stomach tissue with gastritis, atrophy, dysplasia and gastric stromal tumor were significantly increased in model group compared with normal gastric mucosa; and the expression VEGF in gastric tissue with dysplasia was higher than in tissue with inflammation and atrophy (10.8 ± 1.96 *vs* 7.62 ± 0.25, *P* = 0.029; 10.8 ± 1.96 *vs* 6.26 ± 0.76, *P*=0.033, respectively); similarly, expression of VEGF in gastric tissue with gastritis and atrophy has no significant difference (*P* > 0.05); and 3) the expression of VEGF had positive correlation with p-STAT3.

**CONCLUSION**: p-STAT3 plays an important role in gastric cancer formation *via* regulating expression of VEGF to promote the progress of gastric tumor from gastritis.

**Key words:** N-methyl-N'-nitro-N-nitrosoguanidine; Precancerous of gastric cancer; Gastric tumor; p-Signal transducer and activator of transcription 3; Vascular endothelial growth factor; Wistar rat

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**Core tip:** The results show that signal transducer and activator of transcription 3 (STAT3) is partially responsible for the process from chronic gastritis to gastric carcinoma induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and is significantly related the expression of vascular endothelial growth factor (VEGF) in the process, which is considered that STAT3 can induce the abnormal level expres­sion of the VEGF to promote the formation of gastric carcinoma. To the best of our knowledge, this is the first time to report that p-STAT3 is also persis­tently activated from chronic gastritis to gastritis carcinoma induced by administration of MNNG in rats, which is positively associated with the expression of VEGF.

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**INTRODUCTION**

Gastric cancer is the second cause of cancer related death worldwide, and its prevalence rate and mortality are still very high, especially in some developing countries; the high incidence of gastric cancer is a major public health problem in worldwide[1]. Formation and development of gastric cancer are a complex condition associated with multifactorial etiology[2]. *Helicobacter pylori*, high-salt diet, smoking, obesity and Epstein-Barr virus are relative factors which increase gastric cancer risk[3-7]. In addition, environmental factors have a considerable amount of contribution in etiology of cancer[2]. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was used commonly to induce gastric tumor in basic research[8,9], so we also chose MNNG to induce gastric precancerous leision and gastric tumor in this study. Consistent with other tumors, so far, the pathogenesis of gastric tumor is also still not very clear.

Signal transducer and activator of transcription 3 (STAT3), a key transcription factor[10] was showed that plays a vital role in human gastric cancer angiogenesis[11]. It have been reported that activation of STAT3 and the microbial environment were important for gastric tumor initiation and development in the gp130 (757F/F) mouse[11], and it is believed by some investigators that STAT is as a fuel to the fire for the gastric tumorigenesis[12].

Vascular endothelial growth factor (VEGF) plays a very critical role in cancer metastasis because of its function in vascular generation[13,14]. Recently, it was reported to be closely associated with gastric cancer, which may provide additional prognostic information for preoperative evaluation of gastric cancer invasion and tumor type[15], so anti-VEGF was treated as a targeted therapy for treatment of gastric cancer[16]. Several researchers demonstrated the expression of the STAT3 was potentially associated with the expression of VEGF-C in various malignant diseases[17,18], Judd LM *et al*[11] reported that constitutive STAT3 activation promoted VEGF expression and stimulates tumor angiogenesis. But, to our best of knowledge, the roles of STAT3 and VEGF in formation of gastric precancerous lesion and gastric cancer that were induced by MNNG are still unknown.

The aim of this study was to investigat the dynamic expression of p-STAT3 and VEGF as well as the relationship between them in formation of gastric tumor from gastritis induced by MNNG in male Wistar rats.

**MATERIALS AND METHODS**

***Animal preparation***

One hundred and twenty male Sprague-Dawley (SD) rats with 4 weeks old weighing 125-150g were provided by Animal Center of Zheng jiang Chinese medical University. The animals were housed in groups of five per cage under controlled illumination (12:12 h light/dark cycle, lights on/off: 6 h/18 h), humidity (60%) and temperature (22 ± 2℃) for one week to accommodate to the environment. Then the animals were randomly divided into two groups (eath of 60 rats): control group (the rats were able to eat regular food, drink drinking water) and model group (the rats were able to eat regular food, drink drinking water with 100 ug/mL MNNG); each group was randomly divided into other three groups (each of 20 rats): C/M15, C/M25 and C/M40 (15, 25 and 40 digital represent the number of feeding weeks from termination). The Animal Care and Use Committee of the Zhejiang Chinese Medical University approved the protocol.

***Experimental protocols***

Rats were fed by normal food and drinking water or drinking water with MNNG. Rats in C/M15 were sacrified in the end of 15th week in both control group (C15) and model group (M15) and the gastric tissue was collected immediately and stored in -80℃ freeze or was perfused with cold PBS above ice and then was put into a tube with 4% paraformaldehyde for different measurements as described as follows. Similarly, rats in C/M25 or C/M40 were sacrified in the end of 25th week or 40th week and gastric tissue was collected. The whole experimental protocol was described as in Figure 1.

***Measurement of morphological changes***

The rats were terminated respectively in the end of 15th week, 25th week and 40th week. Stomaches were separeated immediately and were opened along long axis on ice to record the conditions as follows: edema, hyperemia, erosion, ulcer or mass, and took photoes by two pathologies who were unaware of the groupings of animals. Then the stomoach tissue was divided into two parts, one was stored in -80℃ low temperature refrigerator and another was put into tubes with 4% paraformaldehyde (PFA).

***Measurement of histology***

Gastric tissue was perfused with PBS followed by 4% PFA in 0.1 M phosphate buffer and cryoprotected in 30% sucrose in PBS overnight at 4°C. Samples were embedded in paraffin wax, and 5 μm specimens were stained with hematoxylin and eosin. The results of histology were divided into four types according the features of histology: chronic superficial gastritis, chronic atrophy gastritis, dysplasia and tumor.

Gastritis is defined by inflammation of the stomach lining associated with mucosal injury. Atrophy gastritis is characterized by chronic inflammatory processes of gastric mucosa that leads to the loss of appropriate glands[19] and gasctric dysplasia is diagonosed according the classification of epithelial neoplasia of the gastrointestinal tract was drafted on the occasion of the World Congress of Gastroenterology in Vienna in 1998[20]; gastric tumor is diagonosed by presence of tumor cell.

***Tissue collection and immunohistochemical processing***

The expression of p-STAT3 and VEGF in gastric mucosa of different groups was evaluated using immunohistochemistry. Specimens were segmented into 1-cm blocks transversely oriented to the hippocampal long axis. Blocks were placed in buffered paraformaldehyde (Sigma, St Louis, MO, USA). After 48 h, specimens were paraffin embedded for immunohistochemistry descriped as Jung HY *et al* and Meng XM *et al*[21,22]. Briefly, endogenous peroxidase activity was blocked using 3% H2O2 in methanol for 15 min. Sections were washed, and stained with a rabbit polyclonal antibody against p-STAT3 and VEGF (Santa Cruz Biotechnology, TX, USA) diluted 1:50. Primary antibody binding was detected using Bond Polymer Refine Detection Kit (Leica, Wetzlar, Germany). The sections were counterstained with hematoxylin, dehydrated and mounted.

***Evaluation of immunohistochemical staining***

Scoring of immunohistochemical results was performed according to the semi-quantitative method. The method was descriped as Meng XM, *et al*[22]. Briefly, the percentage of immunopositive cells was scored using a four-point system: 0 point, < 5% of positive cells; 1 point, 5%-25% of positive cells; 2 points, 26%-50% of positive cells; 3 points, 51%-75% of positive cells; 4 points, > 75% of positive cells. The staining intensity was scored similarly, with 0 point for negative staining, 1 point for weak staining (light yellow), 2 points for moderate staining (brown), and 3 points for strong staining (dark brown). Immunoreactivity score for each lesion was calculated as (the score for the percentage of immunopositivity cells + the score for the staining intensity)/2. Based on immunoreactivity scores, immunohistochemical staining was considered negative (< 0.5), weakly positive (0.5-1.5), or strongly positive (> 1.5).

***Extraction of proteins for Western blot analysis***

Fozen gastric tissue samples (whole layer) were homogenized in homogenization buffer (50mM Tris-HCl, pH 7.2) containing Na3VO4 and a proteaseinhibitor cocktail (Sigma-Aldrich) using an OmniTH homogenizer (OmniInternational, Marietta, GA). After sonication, the homogenate was centrifuged at 2000 rpm for 5 min. The resulting supernatants were collected as total proteins and protein concentrations were measured using the Bio-Rad Protein Assay (Bio-Rad Inc., Hercules, CA)[23].

***SDS-PAGE and Western blot analysis***

Western blots were performed as previously described with minor modifications by Li X *et al*[24]. Briefly, proteins were separated on 12.5 % SDS polyacrylamide gels and blotted onto nitrocellulose membranes. Nitrocellulose membranes were incubated in PBST-milk (0.1 % Tween-20 and 5 % milk), and followed by primary antibodies (2 h) for p-STAT3 and VEGF and β-actin (Santa Cruz Biotechnology, Inc. Texas, USA). Then blots were washed with PBST 3 times, and subsequently incubated for 1h with corresponding fluorescently labeled secondary antibodies (Rockland Immunochemicals, Inc., Gilbertsville, PA) diluted in PBST-milk. The blots were washed 3 times again and the fluorescence intensity of detected protein bands was quantified by the Quantity One system (Bio-Rad Inc., Hercules, CA).

***Statistical analysis***

Statistical analyses were performed using SigmaStat17.0 (SPSS, Chicago, IL, USA). Data was reported as means ± SE. Student’s *t*-test was used to analyze the difference in measurements between the ES group and sham-ES group. Analysis of variance (ANOVA) was applied for multiple comparisons. A *P* value of < 0.05 was considered statistically significant.

**RESULTS**

***Macroscopic and microscopic changes in differen groups***

Administration of MNNG induced significant changes of macroscopy and microscopy in model groups compared with control group. There were little hyperemia and sporadic erosion observed in gastric tissue at the end of 15th week (A15 group); and retuse ulcer, reduction of mucosal folds, gray mucosa and even some mucosal hyperplasias besides hyperemia and erosion were observed at the end of 25th week (A25 group); and the obvious masses could be observed at the end of 40th week (A40 group) (Figure 2A). The respective changes of microscopy were observed in respective groups. There were inflammatory cell observed in samples with gastritis in A15 group, loss of appropriate glands in some samples in A25 group and A40 group and tumour cell in some samples in A40 group (Figure 2B), while those changes were not observed in control group.

***Histological changes in differen groups***

There were only 20% rats with gastritis (4/20) in C15 group, while 42.1% rats with gastritis (8/19), 10.53% rats with atrophy gastritis (2/19) and 5.3% rat with dysplasia (1/19) in M15 group (Table 1). In C25 group, there were 30% rats with gastritis (6/20), 10% rats with atrophy gastritis (2/20) and no rat with dysplasia or tumour; but in M25 group, there were 31.58% rats with gastritis (6/19), 26.32% rats with atrophy gastritis (5/19) and 15.79% rats with dysplasia (3/19) although there was no rat with tumour (Table 1). In the end of study, there were 16.67% rats with gastritis (3/18) in M40 group (*vs* 35% in C40 group, 7/20), 27.78% rats with atrophy gastritis (5/18) (*vs* 20% in C40 group, 4/20) and 27.78% rats with dysplasia (5/18) (*vs* 5% in C40 group, 1/20), the point was that there were 27.78% rats with tumour (5/18) in M40 group (*vs* 0% in C40 group, 0/20) (Table 1).

***Expressions of p-STAT3、VEGF in different pathological type stomach tissue measured by immunohistochemistry***

Expressions of p-STAT3, VEGF in model groups measured by immunohistochemistry were increased significantly compared with control groups. The expressions of p-STAT3 in gastro tissue expressing mainly in the nucleus were showed in Figure 3A, and we found that immunochemistric score of p-STAT3 in tissues with gastritis, tissues with atrophy and tissues with dysplasia were significantly higher than control group (all *P* value less than 0.05) and the expression of p-STAT3 in tissue with dysplasia was higher than in tissue with gastritis and tissue with atrophy (*P* < 0.001, *P* < 0.001, respectively) and the expression of p-STAT3 in tissue with tumor was substantially higher than tissue in control group (*P* < 0.001); but the difference of expression of p-STAT3 between tissue with gastritis and tissue with atrophy was not significant (*P* > 0.05) (Figure 4A).

Similarly, for the expression of VEGF, mainly in the cytoplasm (Figure 3B), was higher in precancerous lesions than control group (*P* < 0.05) showed in Figure 4B. Consistent with p-STAT3, the expression of VEGF in tissue with dysplasia was higher than in tissue with gastritis, tissue with atrophy or control group (*P* < 0.001, *P* < 0.001, respectively); the expression of VEGF in tissue with stromal tumor was substantially higher than tissue in control group (*P* < 0.001) (Figure 4B), but the difference between tissue with gastritis and tissue with atrophy was not significant (*P* > 0.05).

***Expressions of p-STAT3, VEGF in different pathological type stomach tissue measured by Western blot***

To further determine whether the observed p-STAT3 and VEGF consistented with that of changes seen by immunohistochemistry, proteins were extracted from tissue that was scraped from freshly excised stomach, and samples were analyzed with SDS-PAGE Western-blots using primary antibodies for p-STAT3 and VEGF. As shown in Figure 5: (a) both p-STAT3 and VEGF protein expression were significantly higher in tissue with gastritis or atrophy or dysplasis or tumor than control group (*P* < 0.05); (b) both p-STAT3 and VEGF protein expression were significantly higher in tissue with dysplasis or tumor compared with that of tissue with gastritis or atrophy (*P* < 0.05); and (c) there was no significant difference of p-STAT3 and VEGF protein expression between dysplasis and tumor (*P* > 0.05).

***The relationship between expression of p-STAT3 and VEGF***

There was a strong correlation between expression of p-STAT3 and VEGF both in result of immunohistochemistry and Western Blot. In order to further explore the relationship between expression of p-STAT3 and VEGF, we did a reletivitied analysis and found that there was a strong positive correlation of immunohistochemistrical score between expression of p-STAT3 and VEGF (*R*2=0.86, *P* < 0.001) as well as in protein expression measured by Western Blot (*R*2=0.90, *P* < 0.001), suggesting that p-STAT3 may regulate VEGF to promote the formation and development of gastric cancer (Figure 6).

**DISCUSSION**

We performed this study to examine the significance of p-STAT3 and VEGF expression in the process of gastric cancer from gastritis, atrophy gastritis and dysplasia induced by administration of MNNG in rats. The results from this study showed that STAT3 is partially responsible for the process from chronic gastritis to gastric carcinoma induced by MNNG and is significantly related the expression of VEGF in the process. Although overex­pression of p-STAT3 in primary tumor sites has been recognized as a predictor of poorer sur­vival in many malignancies, including gastric cancer[25, 26], to best of our knowledge, this is the first time to report that p-STAT3 is also persis­tently activated from chronic gastritis to gastritis carcinoma induced by administration of MNNG in rats, which is positively associated with the expression of VEGF.

STAT3, plays important roles in the process of inflammation and carcinogenesis as well as tumor metastasis[27, 28]. Normally, STAT3 is activated for a short time and then is deactivated to maintain homeostasis; however, under abnormal conditions, STAT3 activation continues, which triggers oncogene transcription[29]. Only few studies reported the difference of expression of p-STAT3 between normal gastric mucoso and precancerous of gastric cancer. In a clinic study[30], it has been reported that p-STAT3 expression was significantly higher in chronic atrophy gastritis (60.0%), in intestinal metaplasia (77.1%), in dysplasia (68%) and gastric cancer (60.1%) than in normal gastric mucosa (41.1%, *P* < 0.05), consistent with those findings, in our study, we found that p-STAT3 expression in samples with chronic gastritis or atrophy gastritis was significantly higher than that in normal mucosa and was substantially increased in samples with dysplasia or tumor compared with normal mucosa, even higher than that of samples with chronic gastritis or atrophy gastritis, further indicating the potential role of p-STAT3 not only in invasion and metastasis of gastric cancer, but also in the process from chronic gastritis to gastric cancer. However, the interesting thing is that there was no significant difference of expression of p-STAT3 between samples with chronic gastritis and samples with atrophy gastritis, the similar result was observed between samples with dysplasia and samples with tumor, indicating that important effect of p-STAT3 on the progress from atrophy gastritis to gastrical carcinoma.

Vascular endothelial growth factors (VEGFs) are glycoproteins secreted by tumor cells that are the most important factors in angiogenesis and tumor metastasis[31]. The VEGF family includes VEGF-A to F and placental growth factor. It has been reported that VEGF-A and B play a key role in blood vessel growth, whereas VEGF-C and D are important for the growth of lymphatic vessels[32, 33]. The role of VEGFs, particularly VEGF-A, C, and D promote angiogenesis and metastasis of many cancers including gastric cancer (GC)[34].

Some clinic researchs have showed the protential effects of VEGF inhibitor in treatment of multiple cancers including GC[35]. Raica M *et al*[36] investigated the immunohistochemical expression of VEGF on 80 patients with intestinal type gastric carcinoma and found that the reaction for VEGF was positive in 52 from 80 cases (70%), indicating that the expression of VEGF could signify an early angiogenic switch during tumorigenesis. Consistent with the finding, in our study, we also found that the expression of VEGF in samples with dysplasia or tumor was higher than that of samples with chronic gastritis or astrophy gastritis. However, consistent with expression of p-STAT3, both the difference of expression of VEGF between samples with chronic gastritis and samples with atrophy gastritis and the difference between samples with dysplasia and samples with tumor were not significant. According to those results, we analyzed a correlationship between the expression of p-STAT3 and VEGF and found that the expression of VEGF was a significantly positive related with p-STAT3. To our best of knowledge, it is the first time to report the positive relationship between p-STAT3 and VEGF in precancerous leision of gastric cancer induced by MNNG.

Some published papers supported the finding in our study about the relationship between the expression of p-STAT3 and VEGF in gastric cancer. Deng *et al*[37] reported that the STAT3, p-STAT3, and VEGF-D expression in GC tissue was significantly higher than those in adjacent non-tumor tissue, and both STAT3 and VEGF-D mRNA expression was much higher in many GC cell lines than those in GES-1 cell line. With STAT3 siRNA transfection, they found that VEGF-D expression significantly decreased in HGC-27 cell, representing STAT3 potential regulation for the VEGF-D expression[37]. The similar result was observed by Zhu *et al*[38] that (-)-Epigallocatechin-3-gallate could inhibite IL-6-induced VEGF expression and angiogenesis *via* suppressing STAT3 activity in human gastric cancer cell. But the relationship between p-STAT3 and VEGF in precancerous lesion of gastric cancer is still not very clear. In this current study, we found that even in samples with chronic gastritis and atrophy gastritis, both expression of p-STAT3 and VEGF were higher than that of normal mucosa and showed a positive relationship, suggesting that VEGF may be activiated in the beginning of damage of gastric mucosa *via* p-STAT3 pathway induced by MNNG and contribute to the development and progression of GC, highlighting the potential for anti-p-STAT3 as a therapeutic target for prevention of gastric cancer.

In conclusion, STAT3 activation plays an important role in the process of changes in gastric mucosa from chronic gastritis to gastric tumor *via* STAT/VEGF pathway, confirming the potential value of targeted therapy focusing on STAT/VEGF in pretecting precancerous lesion from gastric cancer in clinic applications.

**COMMENTS**

***Background***

Signal transducer and activator of transcription 3 (STAT3) was showed that plays a vital role in human gastric cancer angiogenesis. Vascular endothelial growth factor (VEGF) plays a very critical role in cancer metastasis because of its function in vascular generation. Several researchers demonstrated the expression of the STAT3 was potentially associated with the expression of VEGF-C in various malignant diseases; Judd LM *et al* reported that constitutive STAT3 activation promoted VEGF expression and stimulates tumor angiogenesis. But, to our best of knowledge, the role of STAT3 and VEGF in formation of gastric precancerous lesion and gastric cancer that was induced by MNNG is still unknown.

***Research frontiers***

STAT3 and VEGF have been reported about their some effects on gastric cancer, in this study showed that STAT3 activation plays an important role in the process of changes in gastric mucosa from chronic gastritis to gastric tumor *via* STAT/VEGF pathway.

***Innovations and breakthroughs***

To the best of our knowledge, this is the first time to report that p-STAT3 is also persis­tently activated from chronic gastritis to gastritis carcinoma induced by administration of MNNG in rats, which is positively associated with the expression of VEGF.

***Applications***

It was showed in this study that STAT3 is partially responsible for the process from chronic gastritis to gastric carcinoma induced by MNNG and is significantly related the expression of VEGF in the process, which is considered that STAT3 can induce the abnormal level expres­sion of the VEGF to promote the formation of gastric carcinoma.

***Peer-review***

Previous studies have established that STAT3 has close relationship with VEGF in cancers. However, this is the first time to report that p-STAT3 is also persis­tently activated from chronic gastritis to gastritis carcinoma induced by administration of MNNG in rats, which is positively associated with the expression of VEGF.

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**Table 1 A the histological result**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Group** | ***N*** | **Pathological type** | | | | |
| **Normal** | **Gastritis** | **Atrophy gastritis** | **Dysplasia** | **Tumour** |
| A15 group | | | | | | |
| Control  A15 group | 20  19 | 80.00%(16/20)  42.11%(8/19) | 20.00%(4/20)  42.11%(8/19) | 0  10.53%(2/19) | 0  5.26%(1/19) | 0  0 |
| A25 group | | | | | | |
| Control  A25 group | 20  19 | 60.00% (12/20)  26.32% (5/19) | 30.00%(6/20)  31.58%(6/19) | 10.0%(2/20)  26.32%(5/19) | 0  15.79%(3/19) | 0  0 |
| A40 group | | | | | | |
| Control  A40 group | 20  18 | 40.00%(8/20)  0 | 35.00%(7/20)  16.67%(3/18) | 20.00%(4/20)  27.78%(5/18) | 5.00%(1/20)  27.78%(5/18) | 0  27.78%(5/18) |



F**igure 1 The experimental protocol.** VEGF: Vascular endothelial growth factor; STAT3: Signal transducer and activator of transcription 3.

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# **Figure 2 Administration of N-methyl-N'-nitro-N-nitrosoguanidine could induce macroscopic changes: gastritis, atrophy gastritis, dysplasia and gastric tumor in rats (A) and respective microscopic changes were observed in hematoxylin and eosin staining (B).**

# 

# **Figure 3 Expressions of p-signal transducer and activator of transcription 3, vascular endothelial growth factor in model groups.** A: The expression of p-signal transducer and activator of transcription 3 (STAT3) measured by immunohistochemistry. From normal sample to samples with tumor induced by administration of N-methyl-N'-nitro-N-nitrosoguanidine, the expression of p-STAT3 became more obvious; B: The similiar result was found in the expression of vascular endothelial growth factor.

# 

a

a

a,c

a,c

a,c

a,c

a

a

# **Figure 4 Immunohistochemistrical score of p-signal transducer and activator of transcription 3 and vascular endothelial growth factor.** A: The immunohistochemistrical score of p-signal transducer and activator of transcription (STAT3) was significantly increased by N-methyl-N'-nitro-N-nitrosoguanidine with the progress from gastritis to gastric tumor. The score of p-STAT3 in samples with precancerous lesion or samples with tumor was significant higher than that of normal sample; and the score of p-STATS in samples with dysplasia was significantly higher than that of samples with gastritis or atrophy gastritis, which was comparable with that of samples with tumor; B: The similiar results were found in the score of vascular endothelial growth factor (a*P* < 0.05, *vs* normal group; c*P* < 0.05, *vs* Gastritis or Atrophy Gastritis).

# 

# 

a,c

a,c

a,c

a,c

a

a

a

a

# **Figure 5 Protein expression of p-signal transducer and activator of transcription 3 and vascular endothelial growth factor.** A: The protein expression of p-signal transducer and activator of transcription (STAT3) in samples with dysplasia or tumor was more obvious than others; B: the protein expression of p-STATS in samples with dysplasia or tumor was significantly higher than that of samples with gastritis or atrophy gastritis, but the difference between samples with dysplasia and tumor was not significant, the same result was observed between samples with gastritis and samples with atrophy gastritis; C: The consistent results were found in the protein expression of vascular endothelial growth factor in samples (a*P* < 0.05, *vs* normal group; c*P* < 0.05, *vs* gastritis or atrophy gastritis). AG: Atrophy gastritis.

# 

**Figure 6 Correlation of immunochemistric score between p-signal transducer and activator of transcription 3 and vascular endothelial growth factor.** A: There was a strong correlation of immunochemistric score between p-signal transducer and activator of transcription (STAT3) and vascular endothelial growth factor (VEGF). The immunochemistric score of p-STAT3 was positively related with that of VEGF (*R*2=0.86, *P* < 0.001); B: The similar result was observed in protein expression of p-STAT3 and VEGF (*R*2=0.90, *P* < 0.001).