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The gastric mucosal protective effects of astragaloside IV in mnng-induced GPL rats

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ABSTRACT

Gastric Cancer is one of the most common types of cancer. And the occurrence of gastric carcinoma is an evolutionary histopathological stage. As a result, further research of GPL, which is a borderline of gastric cancer, is indispensable for preventing the formation and development of gastric carcinoma. Several studies have demonstrated a correlation between the expression of autophagy, apoptosis and Gastric cancer (GC). However, the effects of autophagy and apoptosis on human gastric cancer progression, particularly on gastric precancerous lesions (GPL), have not totally been investigated. At present, Astragaloside IV(*AS-IV*) is a saponin purified from Astragalus membranaceous Bge, a traditional Chinese herb that has been widely used for more than 2000 y in the treatment of cancer, cardiovascular and immune disorders. This study was designed to investigate the mechanism of *AS-IV* protecting gastric mucosa in N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced GPL rats. The lesions of GIM and GED were significantly ameliorated compared with the model rats, especially crowded tubular glandular and back-to-back tubular structure, which were the dangerous borderline between GPL and GC. Western Blot analysis showed that the ratio of Bcl-2/Bax and the protein expression of Bcl-XL, p53, Beclin1, p62, ATG5 and ATG12 were decreased and the level of Caspase3 was increased in the group of *AS-IV* compared with the model group; RT-PCR analysis showed that the gene expression Ambra1, Beclin1, ATG5, LC3 and p62 were decreased in the group of *AS-IV* compared with the model group. This research manifested that the occurrence of gastric cancer was preceded by a prolonged precancerous stage, which could be ameliorated by the *AS-IV*. Meanwhile, the mild and moderate stage of precancerous lesions is similar with gastric adenocarcinoma in critical biological processes, including inflammation, cell proliferation, differentiation. But this lesion is very different from cancer, because it does not appear obvious invasion and malignant lesions in this pathologic stag. Further, *AS-IV* could regulate p53 expression to activate the Ambra1/Beclin1 complex in GPL, and it will protect the gastric mucosal injury, prevent and cure gastric mucosal atrophy, intestinal metaplasia and atypical hyperplastic lesions. It provided a potential therapeutic strategy in reversing intestinal metaplasia and dysplasia of gastric precancerous lesions and protecting the gastric mucosa in GPL rats.

1. Introduction

GC is one of the most common types of cancer and it is the second leading cause of cancer-related death worldwide [1]. And the occurrence of gastric carcinoma is an evolutionary histopathological stage, starting with chronic gastritis, followed by GPL, including chronic atrophic gastritis (CAG), intestinal metaplasia, dysplasia, even carcinoma [2,3]. As a result, further research of GPL, which is a borderline of gastric cancer, is indispensable for preventing the formation and development of gastric carcinoma.

Tumor cells are normal epithelial cells that survive by regulating their key proteins to adapt to the microenvironment and eventually develop into cancerous cells that do not depend on growth factors. Previous our studies have found that gastric mucosal epithelial cells have different morphological size and obvious heterogeneity. Within the disorganized gastric mucosal epithelial tissue, enlarged and dilated

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glands were discovered in the dysplastic gastric epithelial cells. A large number of researches have been carried out on gastric cancer, but there is no in-depth study on the mechanism of precancerous lesions.

The previous studies have reported that the dysregulation of p53 increased the risk of gastric cancer. p53, a tumor suppressor, stimulates the expression of apoptosis and autophagy, which combine with Bcl-2 family proteins [4–6]. Numerous studies have reported that abnormal balance of apoptosis and autophagy was related to the occurrence of GC [7]. Autophagy is characterized as both a unique cell-death pathway and an adaptation to stress, supporting tumor cell growth and survival under extraordinary circumstances [8–10]. Apoptosis is a type of programmed cell death, defined by cell membrane blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation [11,12]. As we know that autophagy is a double-edged sword for gastric cancer, in addition, the pro-death (tumor suppressor) and pro-survival (tumor promoter) role of autophagy is particularly associated with its interaction of apoptosis [13–15]. Therefore, it is necessarily elucidated that whether the unbalance between autophagy and apoptosis is an important pathological feature and an early event in the pathogenesis of GPL [16,17]. Ambra1 is an important regulator of the Beclin1 dependent protein of autophagic process [18,19]. Meanwhile, Ambra1/Bcl-2 complex plays an important role in autophagy inhibition and apoptosis induction [20]. In summary, the present investigations focus on protecting gastric mucosa of GPL to reverse gastric carcinoma [21]. With the progression, appropriate intervention of GPL urgently needed.

AS-IV is a saponin purified from Astragalus membranaceus Bge, a traditional Chinese herb that has been widely used for more than 2000 y in the treatment of cancer, immune disorders, cardiovascular and hepatic diseases [22]. Studies have shown that *AS-IV* can reduce the pathological damage of gastric mucosa and atrophy in rats with chronic atrophic gastritis [23]. Emerging evidences have shown that *AS-IV* played an anti-tumor role by inhibiting tumor cell proliferation, anti-tumor angiogenesis, and inducing apoptosis of tumor cells [24]. *AS-IV* possessed the ability to increase expression of the pro-apoptotic genes Bak, Bik and improve expression of the apoptosis markers Bax, cleaved Caspase3 and cytochrome C [25]. Recently, *AS-IV* has exerted anti-HepG2 cell invasion effect by modulating TGF-β/Smads signaling pathway [26]. However, the gastric mucosa protection of *AS-IV* is still obscure in GPL. Numerous reports try to unveil the underlying mechanism of *AS-IV* on GC, but for some of these factors, the signal pathway how they control GPL is fully unknown.

Based on our previous study, we employed GPL model to explore the protective effect of *AS-IV* on gastric mucosal injury in GPL rats induced by MNNG. Meanwhile, we employed GPL model to explore whether *AS-IV* could improve the protective function for gastric mucosa through adjustment of autophagy and apoptosis, which is regulated by the p53.

2. Methods and materials

Astragaloside IV was purchased from Dalian Meilun Biotech Co., Ltd (Dalian, China: N0906A), and its purity was greater than 98% [27]. Kits used for detection of B-cell lymphoma-2 (Bcl-2), B-cell lymphoma-extra large (Bcl-XL), Bcl-2 Associated X Protein (Bax), cysteinyl aspartate specific proteinase-3 (Caspase-3), p-Smad2 and p-Smad3 were purchased from Cell Signaling Technology. The transforming growth factor-βII serine/threonine kinase receptors (TGF-βRII), p53 and RhoA, were purchased from Abcam; E-cadherin, β-catenin, Survivin, Beclin1, p62, ATG5 and ATG12 were purchased from Proteintech; Anti-β-actin was purchased from Sigma-Aldrich. All secondary antibodies (horseradish peroxidase conjugated anti-rabbit IgG and anti-mouse IgG) were purchased from Cell Signaling Technology, Inc.

Gene primer Ambra1, Beclin1, ATG5, ATG12, P62 and LC3 were purchased from Invitrogen Trading (Shanghai) Co., Ltd (Shanghai, China). All other reagents were of the highest grade available commercially.

2.1. Animals and treatment

Adult Sprague Dawley (SD) rats (mean weight was 180–200 g, 44007200027077), 4–6 weeks of age were provided by Guangdong Medical Laboratory Animal Center (No. SYXK (Guang-dong) 2013-0002). All the processes were approved by Animal Ethics Committee of Guangdong Provincie Engineering Technology Research Institute of T.C.M.(GDPITCM160129). Using random number table method, 50 SD rats were randomly divided into normal group (n = 10), the rest were model group (n = 40). Based on the literature and our previous studies, the rat GPL model was set up with minor modifications[28–31]. Briefly, the rats were free drinking MNNG (No. EVNGB-QB; Japan) solution (200 μg/mL) everyday, and were subjected to feed every other day, while the rats in the control group were fed normally. At the eighth, twelfth and sixteenth week, three rats of the model group were randomly sacrificed to perform histological evaluation, respectively. According to the histological evaluation, rats of model group were in the lesion of intestinal metaplasia or dysplasia in the 16th week. Then the model group were randomly divided into three groups: model group, *AS-IV* high dose (*AS-IV* H) group (n = 10, 100 mg/kg) and *AS-IV* low dose (*AS-IV* L) group (n = 10, 50 mg/kg). After treatment for 10 weeks, all rats were sacrificed. All procedures proceeded for 26 consecutive weeks (Fig. 1).

2.2. Reagents

Rabbit anti-TGF-β RII (1:5000, Abcam: ab186838) ; Rabbit anti-RhoA (1:5000, Abcam: ab187027); Rabbit anti-p-Smad2 (1:10000, Cell Signaling Technology: 3108 S); Rabbit anti-p-Smad3(1:10000, Cell Signaling Technology: 9520S); Rabbit anti-Caspase3 (1:1000, Cell Signaling Technology: 9662); Rabbit anti-Bcl-2(1:2000, Cell Signaling Technology: 2876); Mouse anti-Bax (1:2000, Cell Signaling Technology: 2772); Rabbit anti-Bcl-XL (1:2000, Proteintech: 10783-1-AP); Mouse anti-E-cadherin (1:2000, Abcam: ab76055); Rabbit anti-β-catenin (1:2000, Proteintech: 51067-2-AP); Rabbit anti-Survivin (1:2000,

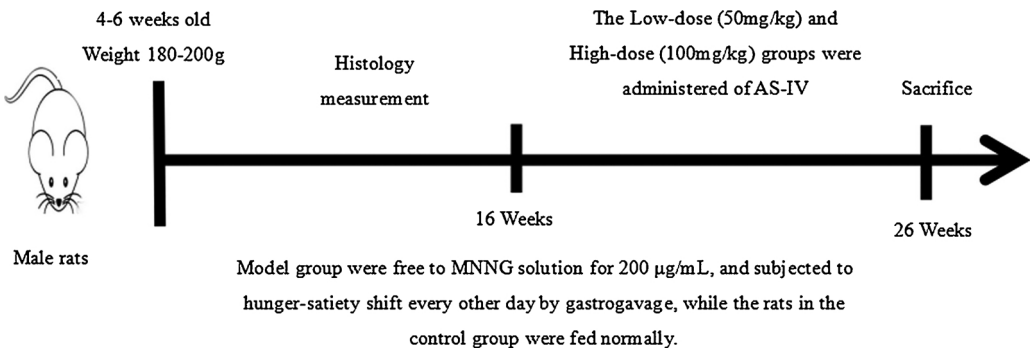

Fig. 1. Scheme of the experimental design(n = 10 rats per group).