Distribution and Expression of Heat Shock Protein 70 in Yak Stomach (Postprint)

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Abstract

This study aimed to investigate the distribution characteristics and differential expression of heat shock protein 70 (HSP70) in yak stomachs. Stomach tissues were collected from 8 healthy adult yaks. Histological staining methods were employed to study cell types within the abomasal glandular interstitium; immunohistochemistry and Western blotting (WB) were used to detect the distribution and expression levels of HSP70 in different stomach compartments. The results revealed: Argentaffin staining and Alcian blue-periodic acid-Schiff (AB-PAS) staining demonstrated that the abomasal glandular interstitium contained numerous endocrine cells. Immunohistochemical detection showed that in the yak rumen, reticulum, and omasum, positive HSP70 expression was observed only in the mucosal epithelial cell layer, with stratum corneum cells exhibiting strong positive expression, while some cells in the basal layer, granular layer, and spinous layer displayed moderate positive expression, and others showed no positive expression; the abomasum exhibited a broader range of positive reactions, with expression present not only in mucosal surface epithelial cells but also in endocrine cells among gastric glands and parietal cells. Moreover, positive reactions were primarily concentrated in the cytoplasm and nucleus of cells. Both immunohistochemistry and WB results indicated that HSP70 expression was highest in the yak abomasum, decreasing sequentially in the rumen, reticulum, and omasum, with highly significant differences among compartments ($P<0.01$). In conclusion, the abomasal glandular interstitium of yaks contains numerous argyrophilic endocrine cells, HSP70 is distributed in all stomach compartments, and its expression in the abomasum is significantly higher than in other stomachs, suggesting that HSP70 may be associated with digestive and absorptive functions in the yak stomach.

Full Text

Distribution and Expression of Heat Shock Protein 70 in Yak Stomachs

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Abstract

This study investigated the distribution characteristics and differential expression patterns of heat shock protein 70 (HSP70) in yak stomachs. Stomach tissues were collected from eight healthy adult yaks, and histological staining methods were used to identify cell types within abomasal glands. Immunohistochemistry and Western blotting (WB) were employed to detect the distribution and expression levels of HSP70 in different stomach compartments. Silver staining and Alcian blue-periodic acid-Schiff (AB-PAS) staining revealed numerous endocrine cells within abomasal glands. Immunohistochemical detection showed that in the rumen, reticulum, and omasum, HSP70 expression was confined to the mucosal epithelial cell layer, with strong positive expression in stratum corneum cells and moderate expression in some cells of the basal, granular, and spinous layers, while other cells showed no expression. The abomasum exhibited a broader positive reaction range, with expression detected not only in surface epithelial cells but also in endocrine and parietal cells within the glands, with positive signals concentrated primarily in the cytoplasm and nucleus. Both immunohistochemistry and WB results demonstrated that HSP70 expression was highest in the abomasum, followed sequentially by the rumen, reticulum, and omasum, with highly significant differences among compartments ($P<0.01$). These findings indicate that the abomasal glands of yaks contain abundant argyrophilic endocrine cells, that HSP70 is distributed across all stomach compartments, and that its expression in the abomasum is significantly higher than in other stomachs, suggesting that HSP70 may be associated with digestive and absorptive functions in the yak stomach.

Keywords: heat shock protein 70; stomach; tissue structure; yak

Introduction

Heat shock protein 70 (HSP70) is a specialized stress protein produced by organisms in response to adverse stimuli. It is ubiquitously present in living organisms and plays crucial roles in regulating cell proliferation and differentiation, anti-apoptosis, stress resistance, and immune response participation [1-3]. In recent years, research on HSP70 in the gastrointestinal tract has provided important clues and evidence for treating gastrointestinal diseases, making it a focus of scientific interest. Currently, studies on HSP70 in the stomach have primarily

concentrated on monogastric animals such as rats [4], mice [5], pigs [6], and humans [7], while its distribution and expression in the stomachs of ruminants remain unclear.

The yak (*Bos grunniens*) [8] is a ruminant species inhabiting the Qinghai-Tibet Plateau region of China that consumes relatively coarse forage, which causes significant damage to the gastric mucosa. Previous research has identified HSP70 as a molecular chaperone in gastrointestinal mucosal cells that provides important protective effects [9]. Therefore, this study employed immunohistochemistry and Western blotting (WB) to examine the distribution characteristics and differential expression patterns of HSP70 in various yak stomach compartments, aiming to further explore the protective role of HSP70 in the yak stomach and provide a theoretical foundation for research on yak gastric metabolism and physiological functions.

Materials and Methods

Sample Collection

In September 2016, eight healthy adult yaks (aged 3-5 years) were selected from a slaughterhouse in Xining, Qinghai, with equal numbers of males and females and body weights ranging from 600-900 kg. Following exsanguination via carotid artery, rumen, reticulum, omasum, and abomasum tissues were rapidly excised, contents were removed, and tissues were rinsed with sterile physiological saline. One portion of tissue samples was immersed in 4% paraformaldehyde phosphate buffer (pH 7.3) for paraffin section preparation, while another portion was rapidly frozen in liquid nitrogen and subsequently stored at -80°C for protein extraction.

Histological Examination and Immunohistochemistry

Fixed tissue samples were processed into 4 m paraffin sections and stained using three methods: (1) Gordon' s silver staining to observe and localize endocrine cells in abomasal tissue; (2) Alcian blue-periodic acid-Schiff (AB-PAS) staining for further observation and localization of secretory cells in abomasal tissue; and (3) immunohistochemical staining using rabbit polyclonal anti-HSP70 antibody (Abcam, UK) diluted 1:1,000, following the SP kit protocol. Phosphate-buffered saline (PBS) (0.01 mol/L) was used instead of primary antibody as a negative control. Sections were counterstained with hematoxylin, dehydrated through an alcohol gradient, mounted, and observed using an Olympus DP71 optical microscopy system.

Western Blotting Analysis of HSP70 Expression

Protein samples were adjusted to uniform concentration, mixed with 4× sodium dodecyl sulfate (SDS) loading buffer, and denatured in a 100°C water bath for 10 minutes before storage at 4°C. After preparing separating and stacking gels,

15 L of protein sample was loaded per well for SDS-polyacrylamide gel electrophoresis. Voltage was switched from 80 V to 150 V when the electrophoresis front reached the interface between stacking and separating gels. Following electrophoresis, gels were ice-bath transferred to polyvinylidene fluoride (PVDF) membranes based on marker bands. Membranes were blocked in 5% skim milk at room temperature for 2.5 hours, then incubated overnight at 4°C with rabbit polyclonal anti-HSP70 primary antibody (Abcam, UK) diluted 1:1,000 in skim milk. After three 10-minute washes with PBST, membranes were incubated for 1.5 hours with goat anti-rabbit IgG secondary antibody (bs-0295G-HRP, Beijing Biosynthesis Biotechnology Co., Ltd.) diluted 1:1,000 in skim milk. ECL exposure solution (Beyotime Biotechnology) (mixed at 1:1 ratio of solutions A and B in darkness) was applied to PVDF membranes, which were then exposed, developed, and fixed using X-ray film in a darkroom for 30 seconds. Dried films were scanned, and β-actin was used as an internal reference. Band expression was analyzed using ImageJ software.

Statistical Analysis

Ten HSP70 immunohistochemical sections were selected from each yak stomach compartment, with ten random fields (1,000×) analyzed per section. Integrated optical density values of HSP70-positive reactions were measured using Image-Pro Plus 6.0 software. Data were analyzed using one-way ANOVA in SPSS 19.0 software and expressed as mean ± standard error (mean±SE). Differences were considered highly significant at $P<0.01$ and non-significant at $P>0.05$.

Results

Histological Characteristics of Yak Abomasum

Silver staining revealed numerous argyrophilic cells distributed among yak abomasal glands with varying staining intensities. Endocrine cells showed strong argyrophilia, appearing black-brown; parietal cells exhibited moderate argyrophilia, appearing brown-yellow; and chief cells showed no argyrophilia and remained unstained (Figure 1 [Figure 1: see original paper]-A). AB-PAS staining demonstrated that some endocrine cells among abomasal glands appeared deep red, parietal cells appeared light red, and chief cells appeared blue (Figure 1-B).

Distribution of HSP70 in Yak Stomachs

Immunohistochemical results showed that in the rumen, reticulum, and omasum, HSP70 expression was limited to the mucosal epithelial cell layer, appearing brown-yellow. Stratum corneum cells showed strong positive expression, while some cells in the basal, granular, and spinous layers showed moderate positive reactions and others showed no expression (Figure 1-C, Figure 1-D, Figure 1-E). In the abomasum, the positive reaction range was broader, with expression detected not only in surface epithelial cells but also in endocrine and parietal

cells within the glands (Figure 1-F). Positive signals were concentrated primarily in the cytoplasm and nucleus. Integrated optical density analysis revealed that HSP70 positive reactions were strongest in the abomasum, followed by the rumen, reticulum, and omasum, with highly significant differences among compartments ($P<0.01$) (Table 1).

HSP70 Expression Levels in Yak Stomachs

Western blotting showed that HSP70 was expressed in the rumen, reticulum, omasum, and abomasum of yaks (Figure 2 [Figure 2: see original paper]). ImageJ software analysis revealed highly significant differences in HSP70 expression among stomach compartments ($P<0.01$), with the highest expression in the abomasum, followed by the rumen, reticulum, and lowest in the omasum (Table 2).

Discussion

In 1987, researchers first identified a class of chromium-staining cells in canine gastric mucosa. Subsequent studies revealed that these cells share common characteristics: they are difficult to identify in hematoxylin-eosin (HE) staining but appear black-brown or brown-yellow with strong contrast after silver staining, leading researchers to hypothesize that these are endocrine cells [10]. As a ruminant, the yak' s forestomach (rumen, reticulum, omasum) serves a mechanical digestive function, while only the abomasum contains abundant secretory cells among its glands and functions as a true digestive stomach. Therefore, this study used silver staining to identify cells among yak abomasal glands and found numerous argyrophilic cells. Endocrine cells appeared black-brown, parietal cells brown-yellow, and chief cells showed no argyrophilic staining, similar to findings in camel gastric fundic glands [11]. This staining method identified secretory cells among yak abomasal glands and laid the foundation for subsequent identification of immunohistochemical positive cell types.

Yaks consume relatively coarse forage that strongly stimulates and damages forestomach mucosal epithelium. As one of the fastest proliferating, differentiating, and apoptotic tissues in the body, gastrointestinal mucosal epithelium exhibits rapid cell damage repair [12-13]. Studies have shown that different cells within the same tissue undergo asynchronous proliferation, differentiation, and apoptosis cycles [14]. Alwajeeh et al. [4] and Omar et al. [5] demonstrated that HSP70 inhibits apoptosis in rat gastric tissue cells, providing important gastric protection. Research indicates that under normal physiological conditions, HSP70 expression is low in human, mouse, and rat gastric mucosa but increases significantly after injury stimulation [15-18]. This study' s immunohistochemical and WB findings revealed strong HSP70 positive reactions in the stratum corneum of yak forestomach, suggesting a role in inhibiting excessive mucosal cell apoptosis, accelerating cell repair, and protecting gastric mucosa. Moderate positive reactions in some cells of the basal, granular, and spinous layers, with no expression in other cells, may relate to asynchronous cell proliferation,

differentiation, and apoptosis cycles. Notably, HSP70 expression was highest in the rumen, followed by the reticulum, and lowest in the omasum. As the first stomach compartment in ruminants, the rumen serves as the first line of defense against food stimulation, with the highest degree of mucosal keratinization and greatest damage [19]. We therefore hypothesize that differential HSP70 expression in yak forestomach may correlate with mucosal damage severity, consistent with findings by Donnelly et al. [15] and Silva et al. [16] showing that more severe injury stimuli result in higher HSP70 expression.

Studies have confirmed that gastric endocrine cells not only have secretory functions but also regulate secretion in other cell types. Their secreted histamine plays a crucial regulatory role in stimulating parietal cell acid secretion [20-21]. Wada et al. [22] found that rat gastric acid can induce gastric mucosal heat shock protein 72 (HSP72) expression, thereby reducing acid-induced mucosal damage. Abnormal gastric pH and acid-base imbalance are major causes of gastric ulcers, and the stomach may induce HSP70 expression through self-regulatory mechanisms to maintain acid-base balance and enhance ulcer healing [23-24]. Arora et al. [25] demonstrated that endogenous antioxidant enzyme elevation and lipid peroxidation during mouse gastric ulcer injury lead to HSP70 upregulation for mucosal protection. Marruchella et al. [6] found HSP70 expression in porcine esophageal mucosal epithelial cells, protecting esophageal mucosa from damage. This study' s immunohistochemical and WB results showed that in yak abomasum, HSP70 was expressed not only in surface epithelial cells but also in endocrine and parietal cells within glands. We hypothesize this may protect abomasal mucosa from damage and regulate secretory functions of endocrine and parietal cells, thereby maintaining mucosal integrity, regulating gastric acid-base balance, and promoting food digestion and absorption. The significantly higher HSP70 expression in abomasum compared to forestomach may relate to its broader positive distribution pattern. Notably, HSP70 showed no positive reaction in abomasal parietal cells, a finding requiring further investigation.

Conclusion

1. Yak abomasal glands contain abundant secretory cells.
2. In yak forestomach, HSP70 shows strong positive reactions in stratum corneum cells and moderate positive expression in some cells of the basal, spinous, and granular layers, with no expression in other cells.
3. The abomasum exhibits a broader positive reaction range, with expression detected not only in mucosal epithelial cells but also in endocrine and parietal cells among the glands.
4. HSP70 is expressed in all four stomach compartments of yaks, with highest expression in the abomasum, followed by the rumen, reticulum, and lowest in the omasum.

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