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Histological Structure and Mast Cell Distribution of Hedgehog's Small Intestine

Youbao Zhong[1], Xianlai Zhang[1], Xiaofen Hu[1] and Yong Li[1, 2,*]

[1]*College of Animal Science and Technology, Jiangxi Agricultural University, No. 1101 Zhimin Avenue, Economic and Technological Development District, Nanchang 330045, Jiangxi, P.R. China*

[2]*Jiangxi Provincial Key Laboratory for Animal Health, College of Animal Science and Technology, Jiangxi Agricultural University, No. 1101 Zhimin Avenue, Economic and Technological Development District, Nanchang 330045, Jiangxi, P.R. China*

ABSTRACT

The small intestine is the major site of animal digestion and absorption; however, little is known about the morphological characteristics of the hedgehog's small intestine. This study was conducted to investigate the microstructure of the hedgehog's small intestine and mast cell distribution by conventional HE and modified toluidine blue staining, respectively. Despite its similarity to the general mammal small intestine microstructure, the present results indicated that the hedgehog duodenum contained fewer Brunner's glands, and that the Paneth cells were lacking in the crypt. The connective tissue was denser in the jejunal submucosa, but the muscularis was thicker. The intestinal glands were greatly developed in the ileum, lymphoid nodules were observed in the mucosa, and diffuse lymphoid tissue was distributed in the lamina propria. Meanwhile, goblet cells gradually increased from the duodenum to the ileum. The mast cells showed various shapes and sizes in the small intestine and could be detected at four layered structures in the small intestine, but were mainly distributed in the lamina propria and the submucosa, surrounded with connective tissue and blood vessels. In conclusion, the mast cells, goblet cells, lymphocytes and lymphoid nodules contributed to the small intestine mucosal immune barrier, and the distribution characteristics of the mast cells demonstrated great significance for the hedgehog's immune system.

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YL and YZ conceived and designed the study. XZ and XH analysed the data. YL and YZ wrote the manuscript.

Key words
Hedgehog, Small intestine, Microstructure, Mast cells.

INTRODUCTION

The hedgehog is a type of wild animal (Zhang and Wang, 2006), mainly inhabiting ravine, farmland, and bush and is widely distributed in three north provinces, specifically the Inner Mongolia, the Yangtze River Basin and the Shaanxi-Gansu area of China. The hedgehog has traditional Chinese medicinal purposes (Zhao *et al*., 2007) and great edible value, as its meat contains a high content of unsaturated fatty acids that are easily absorbed by the human digestive system (Li, 2009). The hedgehog can be used as an experimental animal in hibernation physiology studies. Increasingly exploited in recent years, the hedgehog has begun to receive attention as an important economic asset. Microstructure investigation of the small intestine is helpful to further understand its characteristics and function.

Mast cells distribute in a wide range of mammalian tissues, such as blood vessels, nerves, epithelium and smooth muscle, and may release pro-inflammatory cytokines, including cytokines and growth factors (Galli *et al*., 1999). Mast cells (MCs) in different species and organs can be divided into mucosal MCs and connective tissue MCs (Miller and Pemberton, 2002). MCs play an important role in granular cells during these pathological processes, such as immediate and delayed hypersensitivity, arthritis, multiple unexplained inflammation and fibrosis, and inflammatory bowel disease (Theoharides and Cochrane, 2004). MCs contain numerous basophilic granules, which are composed of 5-serotonin, histamine, heparin and other bioactive substances, and exert great function for animal health and disease prevention. MCs take part in innate immunity related to bacterial infections and show a direct relationship with viral arthritis (Liu *et al*., 2014). MCs contain Fc receptors at their surface and show a significant effect in IgE-related reactions (Frangogiannis *et al*., 1999). The immune mechanism has become the focus of many studies for more than 100 years. The hedgehog is a precious species in the wild environment; identifying the distribution of its small intestine MCs provides a reference value for the immune mechanism of mammalian feeding tests in the wild, as well as clean or sterile environments, and it shows special significance for the intestinal mucosal immune barrier.

* Corresponding author: liyong2912@163.com
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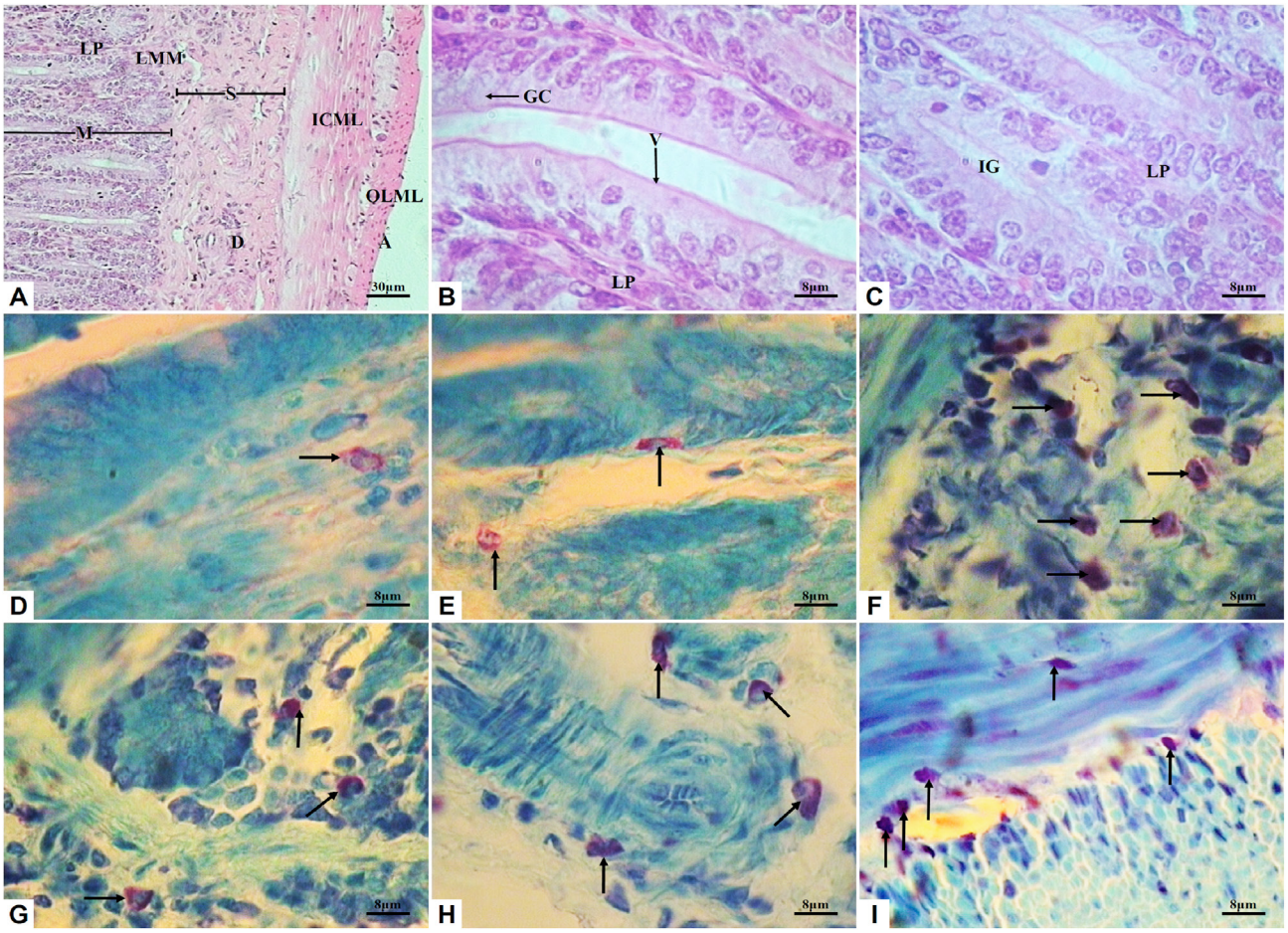

Fig. 1. Microstructure and mast cells in the hedgehog's duodenum (A, magnification of 100X; B to I, magnification of 400X). **A to C,** HE staining; **D to I,** toluidine blue staining. Black arrows indicating MCs. M, mucosa; LMM, lamina muscularis mucosa; LP, lamina propria; GC, goblet cell; A, adventitia; ICML, inner circular muscle layer; OLML, outer longitudinal muscle layer; S, submucosa; D, duct; V, villi; IG, intestinal glands.

MATERIALS AND METHODS

Animals and tissue preparation

Healthy wild hedgehogs were anaesthetized to cut the carotid artery and were quickly necropsied to open the abdominal cavity. Small intestines, including the duodenum, jejunum and ileum, were immediately removed and cleaned using 0.01 M PBS, followed by fixing in a 4% polyformaldehyde solution for 24 h. All of the procedures were performed in accordance with the Animal Care and Welfare Committee and Guidelines of Animal Experiments of our institute throughout the experiment.

Histological studies

The intestines were processed for histological studies according to conventional procedure. The fixed, dehydrated tissues were embedded in paraffin, and then sectioned at a 5μm thickness. Some sections were treated by conventional HE staining. In addition, after other sections were deparaffinized, they were stained for 30s in modified toluidine blue solution, washed in distilled water two times (5 min each), separated from excess colour in a 90% ethanol solution, dehydrated in 100% ethanol two times (5 min each), made transparent in xylene, and mounted with neutral resin.

The small intestine microstructure and distribution of MCs was examined under an Olympus light microscope. Five slices were selected and evaluated for each example, and the corresponding positive photographs were taken by using two-dimensional image measurement software.

RESULTS

Microstructure of the hedgehog's small intestine

The intestinal wall of the duodenum, jejunum and ileum from the hedgehog can be divided into

mucosa, submucosa, muscular, and adventitia layers. The duodenal submucosa is especially prosperous and mainly composed of dense connective tissue containing few Brunner's glands. The muscular layer is constructed with inner circular muscle and outer longitudinal muscle, but the inner circular muscle is thicker than the outer longitudinal muscle. The serous membrane basically consists of mesothelium and a thin layer of connective tissue (Fig. 1A). Intestinal microvilli are developed with a thicker epithelial monolayer and fewer goblet cells, and lymphocytes tend to move into the intestinal cavity from the monolayer epithelium (Fig. 1B). Intestinal glands in the lamina propria belong to simple tubular gland; its basement is primarily composed of columnar cells and a few goblet cells, but Paneth cells are not observed in the crypts (Fig. 1C). Submucosa in the jejunum is almost completely composed of dense connective tissue; its thickness is similar to the inner circular muscle layer. The adventitia is a thin layer of connective tissue (Fig. 2A). Most intestinal glands are developed into simple tubular glands with more goblet cells (Fig. 2C).

Intestinal glands are also developed in the ileum; the lamina propria contains rich, dispersive lymphoid tissue. The developed submucosa comprises a large number of ducts, blood vessels and dispersive lymphoid tissue (Fig. 3A). In mucosa, glands abnormally develop into tubular and branched tubular glands that are connected by dense connective tissue, and the lymphoid nodule or tissue mainly exists in the lamina propria (Fig. 3B). Many goblet cells are densely distributed in the layer of mucosa almost without villus, and the epithelial cells are heavily basophilic and dyed with dark blue (Fig. 3C).

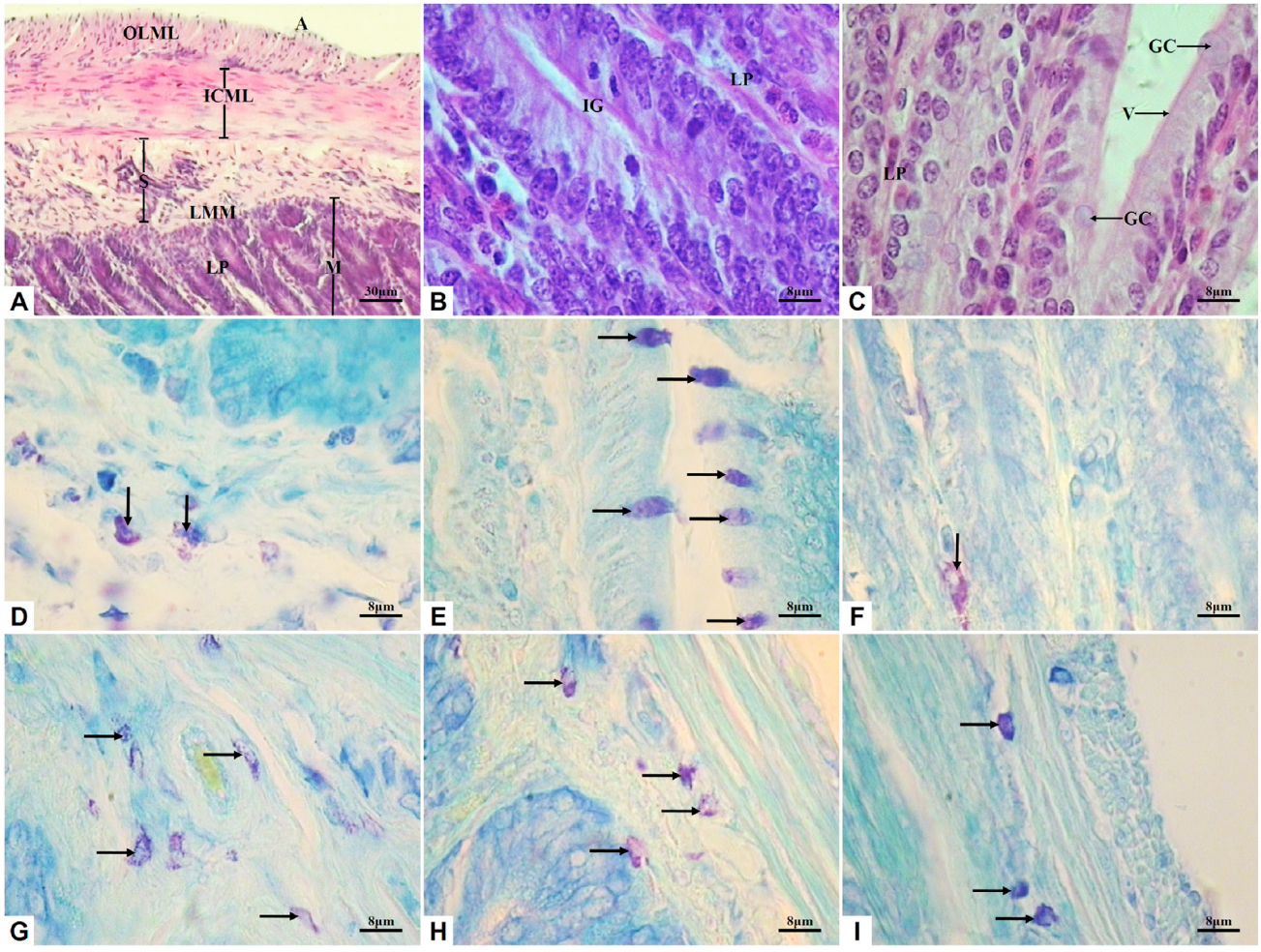

Fig. 2. Microstructure and mast cells in the hedgehog's jejunum (A, magnification of 100X; B to I, magnification of 400X). **A to C,** HE staining; **D to I,** toluidine blue staining. Black arrows indicating MCs. M, mucosa; EM, epithelium mucosa; LMM, lamina muscularis mucosa; LP, lamina propria; GC, goblet cell; A, adventitia; ICML, inner circular muscle layer; OLML, outer longitudinal muscle layer; S, submucosa; V, villi; IG, intestinal glands.

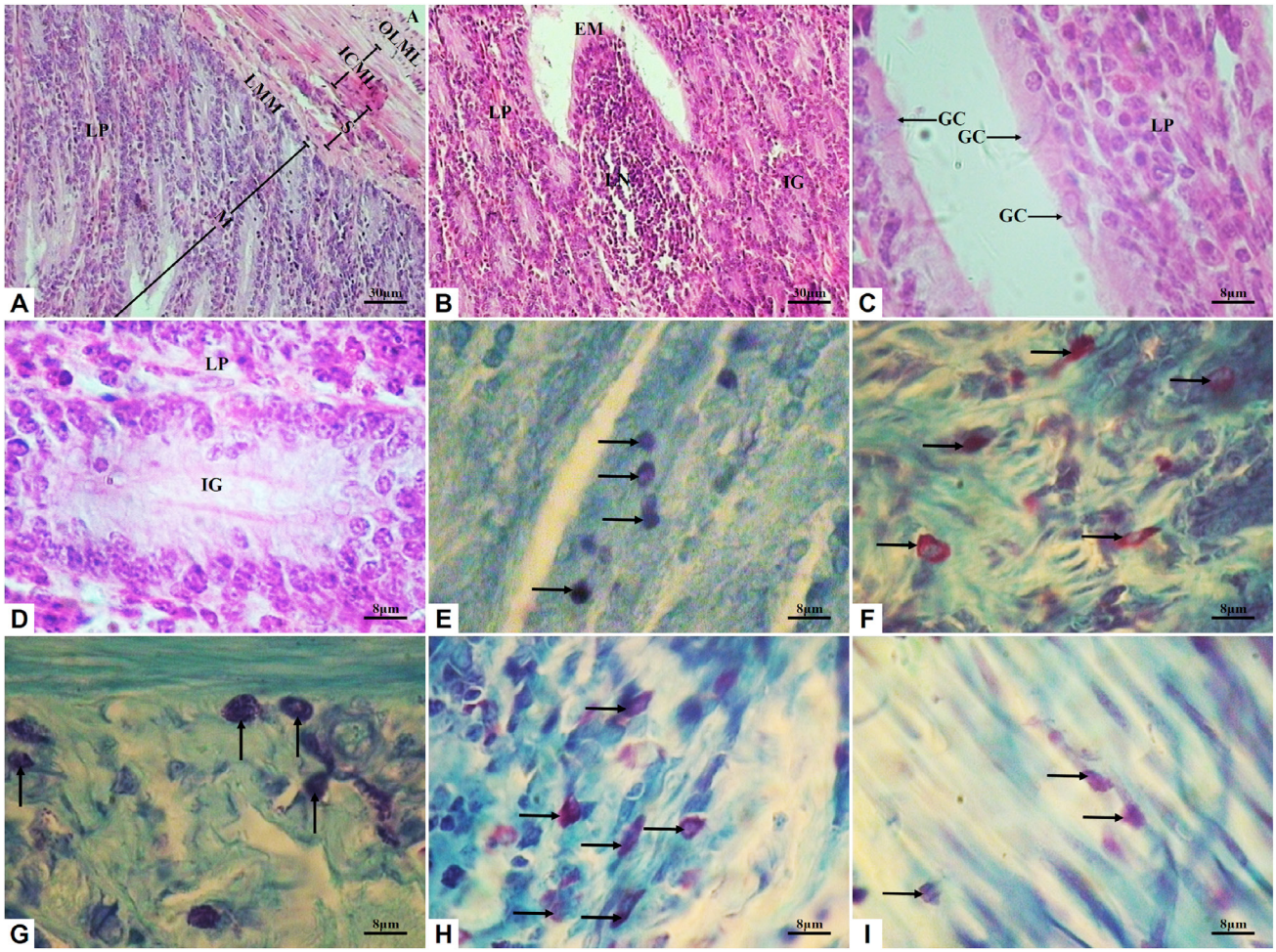

Fig. 3. Microstructure and mast cells in the hedgehog's ileum (A and B, magnification of 100X; C to I, magnification of 400X). **A to D,** HE staining; **E to I,** toluidine blue staining. Black arrows indicating MCs. M, mucosa; LMM, lamina muscularis mucosa; LP, lamina propria; GC, goblet cell; A, adventitia; ICML, inner circular muscle layer; OLML, outer longitudinal muscle layer; IG, intestinal glands; LN, lymphoid nodule.

Distribution characteristics of MCs in small intestine

Positive MCs in the small intestine showed purple-red as they were modified with toluidine blue staining; the light blue nucleus was located at the centre of cytoplasm and filled with dense purple particles. MCs were present at various volume sizes and shapes, such as long strip, fusiform, round, oval or irregular shape. MCs were found in the intestinal wall of the duodenum, jejunum and ileum and were mainly distributed in blood vessels of the submucosa and lamina propria of the mucosa. In the duodenum, MCs were observed at the lamina propria (Fig. 1D), among the intestinal glands (Fig. 1E), within the submucosal connective tissue (Fig. 1F, G) and near the artery (Fig. 1H) and muscle layer (Fig. 1I). MCs were visible at the mucous epithelium (Fig. 2D), lamina propria (Fig. 2F), near the submucosal blood vessels (Fig. 2G) and fibre (Fig. 2H), and the muscle layer (Fig. 2I) in jejunum. Moreover, MCs were also found at the lamina propria (Fig. 3E), submucosal connective tissue (Fig. 3G), aggregated in lymphatic nodules (Fig. 3H), and within the inner circular muscle layer (Fig. 3I) in the ileum. The jejunal goblet cells were stained with purple-blue granules (Fig. 2E), but the goblet cells were faintly visible in the duodenum.

DISCUSSION

Structure of small intestine facilitates adaptability to the hedgehog's function

The small intestine is the major site of animal digestion and absorption, and its structure plays a key role in digestion and absorption, directly determining the efficiency of nutrient and energy absorption from

food. Fluid secreted by the duodenal glands enters the intestinal tract to promote the absorption of nutrients in the duodenum. These glands were extremely few in the submucosa of the hedgehog's duodenum, which may lead to insufficient nutrient absorption; even their faeces contain some nutrients. The intestinal villi can increase the area in contact with the food (Yang *et al*., 2013); goblet cells can secrete mucus (Feng and Nurguli, 2012) to accelerate gastrointestinal peristalsis and promote faecal discharge, also have some important defense mechanism against irritants, microbial attachments and invasion (Arman and Ucuncu, 2017). The longest duodenal villi contribute to higher absorption efficiency than that of the jejunum and ileum. There are no branching phenomena in the intestinal villi, which is also observed in the Oriental White stork (Jia *et al*., 1990) and Red Crowned Crane (Zhang *et al*., 1999). Abnormally developed ileal glands and a large number of goblet cells can promote ileal digestion and absorption function by secreting mucus. They are also directly related with the hedgehog's food intake habits. The hedgehog chiefly consumes crickets, worms, melon and other fruits with high water content, as these foods are likely to be crushed and easily digested and absorbed. The integral structure of the small intestine is similar to other common mammals, such as the dog, cat, horse and pig, but some differences also exist among their structures. Canine Brunner's glands are rich, and the unit cavity that secretes mucus is large and lined with tall columnar cells; feline villi are long, and rich Brunner's glands enter the muscularis mucosa and divide into many independent lobules; equine Brunner's glands are both mucoid and serous, but the secretory cavity is very small; the porcine duodenal gland also extends a significant length in the jejunum (Huang, 1990; William and Chen, 2007; Wang, 2002). It is indicated that the structural differences are adaptable to the hedgehog's function of its small intestine.

The hedgehog contains rich lymphoid tissue in its small intestine, and diffuse lymphocytes or lymphoid nodules are widely distributed in the epithelium and lamina propria, which together constitute the biological basis of mucosal immunity. Intestinal intraepithelial lymphocytes are not only members of the immune system; they are one of the prime immune cells in contact with pathogenic microorganisms but also one of the earliest cells involved in immune response, during which they secrete IL-2, IL-5, IFN-r and other cytokines. Intestinal intraepithelial lymphocytes have cytolytic activity and can kill not only target cells that T lymphocytes cannot kill but also target infected or cancerous epithelial cells due a variety of factors, and therefore, play a role in immune surveillance (Zhang, 2012). The structure characteristics probably lead to the adaptability of the hedgehog's immune function of its small intestine.

Number of MCs contributed to intestinal immune level

MCs mainly distribute in tissues directly in contact with the external environment, such as animal skin, the gastrointestinal tract, respiratory tract mucosa, lymphoid tissue and perivascular connective tissue (Metcalfe *et al*., 1997). MCs can not only enhance the specific immune response but can also be used as effector cells that are involved in the innate and adaptive immune responses (Stenton *et al*., 1998). The hedgehog faces more exogenous disease since it lives in a wild environment; MCs exert a significant role in fighting against these diseases and eliminating pathogens via phagocytosis (Liu, 2012). The small intestine is the contact site that is directly related to exogenous pathogens and usually becomes vulnerable to the invasion of pathogenic microorganisms. Many MCs located in the small intestine can significantly resist the diffusion of pathogenic microorganisms; therefore, the MCs assume the primary defence line of natural immunity. When the body is invaded by a pathogen, MCs are activated and initiate a process similar to oxidation sterilization of neutrophils and macrophages, which can conduct phagocytosis and degradation of the pathogen (Farhadi *et al*., 2007). These MCs assume defence roles in the intestinal tract and can avoid disease expansion during infection. MCs participate in immune functions caused by vascular responses due to histamine secretion into blood vessels, which can lead to enhanced or inhibited immune functioning (Hugjiltu, 2004). Capillaries are rich in the intestinal mucosa, and MCs contain histamine, as well as other biological effector molecules, and primarily distribute around the blood vessels, facilitating histamine absorption into the blood vessels. Jejunal goblet cells were stained purple-red, indicating that they contain heparin and histamine, *etc*., and take part in the mucosal immune response of the intestine. While facing pathogens from outside, MCs can secrete many biologically active immune substances, leading to enhanced resistance to pathogens. MCs, goblet cells, the lymphoid nodule, and intestinal epithelial lymphocytes construct the hedgehog's mucosal immune barrier to maintain its good condition (Zhang *et al*., 2013). The number of MCs in local tissue probably reflects its immune level to some extent (Xue *et al*., 2011). In conclusion, the wide distribution of MCs in each intestinal layer contributed to the special immune mechanism for the hedgehog.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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