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RESEARCH ARTICLE

Immunohistochemical Expressions of Two Intermediate Filaments in the Rumen of Goat

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ABSTRACT

To investigate the variation in expression of vimentin and desmin in the rumen of goat by analyzing the types and distribution of positive cells, rumen tissue fixed in formalin and embedded in paraffin was used that had been sourced from adult (10-month-old) goats. The rumen tissue was stained by immunohistochemical labeling with antibodies against vimentin and desmin. Vimentin-immunoreactivity (Vim-ir) was extensively expressed in the cells with some processes of the lamina propria of ruminal papillae and the subepithelial region. However, the positive cells of lamina propria of ruminal papillae appeared with thin biopolar (spindle-shaped) cytoplasmic processes. In the lamina propria, Vim-ir was found in the fibroblasts or fibroblasts-like cells, which have longer processes. While, within the muscular layer, it was also strongly localized in the cells of interstitial space between the smooth muscle bundles and these cells were observed satellite-shaped with some thin processes. In addition, Vim-ir was also noted in glial cells of ganglion and nerves predominantly in the endoneurium and around the perineurium. In contrast, desmin-immunoreactivity (Des-ir) was only noted within the smooth muscle cells of rumen. This study first time reported the expression of vimentin and desmin within the rumen of goat, which may play a critical role in the physiological processes and prove to be helpful in future studies to compare different abnormal conditions/diseases in the rumen of goat.

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INTRODUCTION

The rumen is the largest chamber within the forestomach of ruminant and located in the first and forefront part. It can produce rhythmic contraction to move and mix ingested feed for digestion, eliminate gases through eructation and send food particles back to the mouth for re-mastication (Illius *et al*., 2000; France, 2013). While, the cytoskeleton is the main component in the rumen which is essential for contraction, providing mechanical support to different cells and it is conducive to normal growth regulation, maturation, differentiation, division, integrity and function of tissue (Eriksson *et al*., 2009). The cytoskeleton helps in maintaining the internal communication and morphological structure of the cell, and also participates in cellular movement and intracellular material transportation. Their profile frequently changes according to physiologic needs, and in response to enormous pathologic conditions (Eriksson *et al*., 2009; Nekrasova *et al*., 2011; Xia *et al*., 2013; Capetanaki *et al*., 2015).

Intermediate filaments (IFs) are the product of a mulfigeae family, it is considered to be the main member of the cytoskeleton within every eukaryotic cell. IFs are the most complicated cytoskeletal element because it contains several types of different polypeptides (Köster *et al*., 2015). IFs expression is regulated in a spatial-temporal and tissue-specific pattern in different animal. Vimentin is one of the major classes of the IFs, and is especially expressed in the cells of the mesenchymal origin. Recently, vimentin has been implicated in development and regeneration of gastrointestinal tissues (Eriksson *et al*., 2009). Desmin filaments are potent marker of muscle cells and mainly expressed in various muscle types. Absence of desmin filaments in the muscle cells leads to functional and structural defects (Weisleder *et al*., 2004). It is found that the expression of IFs changed greatly under certain pathological conditions,

either up-regulation or down-regulation (Bayo *et al*., 2015). However, the expression of IFs (vimentin and desmin) in the rumen of goat is still not reported. In this study, vimentin and desmin antibody was used for immunohistochemistry to determine the distributional characteristics of the two IFs in the rumen of goat.

MATERIALS AND METHODS

Five adult goats (10-month-old) of the either sex were obtained from a commercial farm. Rumen samples were removed from these goats right after euthanasia. Then immunohistochemical staining was performed according to the manufacturer's recommendations and as suggested in previous studies (Obert *et al.*, 2007; Eid *et al*., 2013). Samples were immediately washed using 0.1 mol/L phosphate buffered saline (PBS) (pH 7.3) and then fixed in the 10% neutral buffered formalin for overnight. Underwent the dehydration by gradient ethanol, the samples were processed for embedding in paraffin. Paraffin sections were cut at a thickness of 4-5 µm and placed on poly-L-lysine-treated glass slides. Immuno-histochemical studies were executed on prepared sections underwent deparaffinization and covered with 3% hydrogen peroxide in PBS for 15 min at 37°C to block the further activity of endogenous peroxidase. Then the sections were pre-incubated with 5% bovine serum albumin (BSA) in PBS for 1 hour at 4°C. Then incubated with anti-vimentin (1:150) polyclonal rabbit antibody (Boster Bio-Technology, Wuhan, China) and anti- desmin (1:100) polyclonal rabbit antibody (Boster Bio-Technology, Wuhan, China) for 24 hours at 4°C. After washing, the sections were incubated with biotinylated anti-rabbit IgG secondary antibody (Boster Bio-Technology, Wuhan, China) for 1 hour at 37°C. After peroxidase blocking, the sections incubated again with avid in-biotinylated peroxidase complex for 45 minutes at 37°C. The chromogen/substrate was revealed using DAB (Boster Bio-Technology, Wuhan, China). Finally, photographs were taken at 40-400 magnification for microstructure analysis under microscope (Olympus BX53, Tokyo, Japan).

RESULTS

The vimentin-immunoreactivities (Vim-ir) were widely distributed in the various area of the rumen tested. Vim-ir was extensively expressed in the cells with some processes of the lamina propria of ruminal papillae and the subepithelial region (Fig. 1 A, B, 2 B). The positive cells of the subepithelial region show a cellular body (nuclear or somatic region) and multipolar (dendritic-like) cytoplasmic processes, and their processes seem extended into the epithelial tissue. However, the positive cells of lamina propria of ruminal papillae show thin biopolar (spindle-shaped) cytoplasmic processes (Fig.1C). The cellular body presents either elongated or triangular morphology and contains an elongated, ovoid nucleus (Fig. 1D), and a thin layer of cytoplasm (somatic cytoplasm). The cytoplasmic processes communicate with those of neighboring positive processes (Fig. 1C, D). In the lamina propria, Vim-ir was found in the fibroblasts or fibroblasts-like cells (longer and thinner processes) (Fig. 2A, B, C, D). And the processes of positive cells tend to be arranged in close proximity to collagen fiber bundles (Fig. 2C, D). Vim-ir was also labeled in the endothelial cells of blood vessels, both on arteries (Fig. 3A) and veins (Fig. 3B). In addition, Vim-ir was also widely distributed around smooth muscles of vessels (Fig. 3). In the muscular layer, it was also strongly stained in the cells of interstitial space between the smooth muscle bundles and these cells show satellite-shaped with some thin processes (Fig. 4A). Intercommunicating processes of positive cells surround fascicles of smooth muscle cells, forming a dense reticular network with a particular spatial arrangement (Fig. 4B). In addition, Vim-ir is present in glia cells of ganglion and nerves predominantly in the endoneurium and around perineurium (Fig. 5A). In the subserosa, Vim-ir was labeled in the fibroblasts and endothelial cells of blood vessels (Fig. 5B). In contrast, desmin-immunoreactivity (Des-ir) was expressed only in the smooth muscle cells (Fig. 6A, B), and absent in lamina propria (Fig. 6C) and the interstitial space between the smooth muscle bundles (Fig. 6D).

DISCUSSION

Unlike plants, animal cells lack cell walls and, therefore, IFs constitute a major structural element of animal cells for mechanical support. IFs are able to participate in transportation of stress or disease-induced signaling molecules in order to adapt the regulation of survival signaling. Because of this, IFs are widely used today in histology and histopathology serving as markers of some cell type. Our results are in general agreement with the studies reported in most of the various organs of animal species in terms of distribution of cytological types about vimentin and desmin filaments in the mouse (Kachinsky *et al*., 1995), budgerigar (Bavdek *et al*., 1997) and human (Michalczyk *et al*., 2001). These studies have revealed that the vimentin and desmin, are accepted as two primary members of IFs, mainly expressed in mesenchymal originated cells and three types of muscle tissues, respectively.

In the present study, Vim-ir shows a strong positive expression in the endothelial cells of vessels, while it was negative in smooth muscle cells of vessels. These results were not consistent with the previous findings in the moulting skin of budgerigar (Bavdek *et al*., 1997), human breast (Michalczyk *et al*., 2001) and rat uterus during implantation (Korgun *et al*., 2007). They thought that Vim-ir was present in the endothelial cells, fibroblasts and smooth muscles of blood vessels, however smooth muscles of blood vessels are negative in our findings. This contradiction may due to previous research on developmental and secretary tissues, due to physiological nature of these organs some vimentin expression maybe activated, so we speculate that the vascular smooth muscle remains positive for Vim-ir.

Vim-ir in the lamina propria of rumen was still not reported. The cellular body presents either elongated or triangular morphology and contains an elongated or ovoid nucleus along with thin layer of cytoplasm (somatic cytoplasm). The cytoplasmic processes communicate with those of neighboring positive processes or other cells.

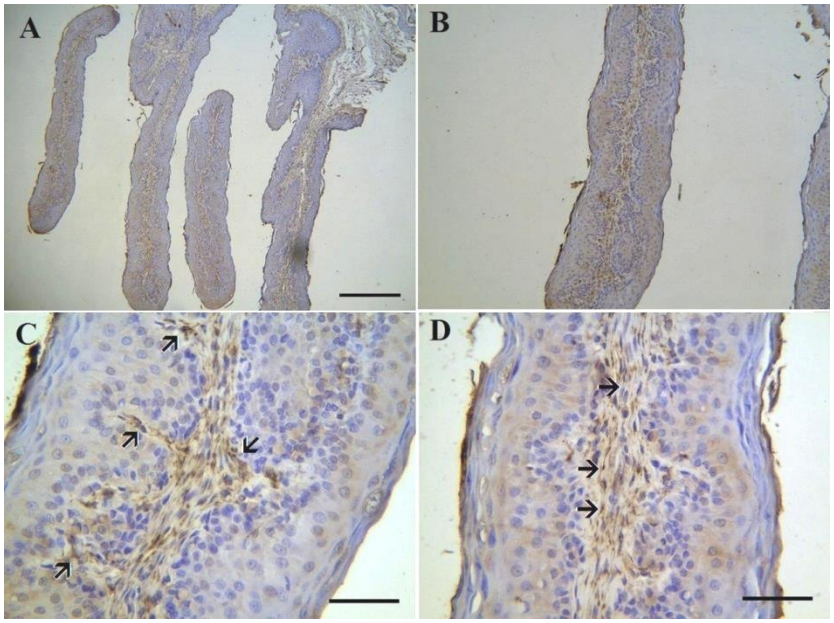

Fig. 1: Photomicrograph showing the Vim-ir in the ruminal papillae. (A) Vim-ir are widely distributed in all ruminal papillae. (B) A higher magnification view of Fig. A shows the Vim-ir are very dense in the subepithelial region and lamina propria. (C) A higher magnification view of Fig. B shows the positive cells of the subepithelial region and a multipolar (dendritic-like) cytoplasmic processes (long arrow). (D) A higher magnification view of Fig. B shows the positive cells of lamina propria of ruminal papillae with thin biopolar (spindle-shaped) cytoplasmic processes (long arrow). Scale bars: A: 500µm; B: 200µm; C, D: 50µm.

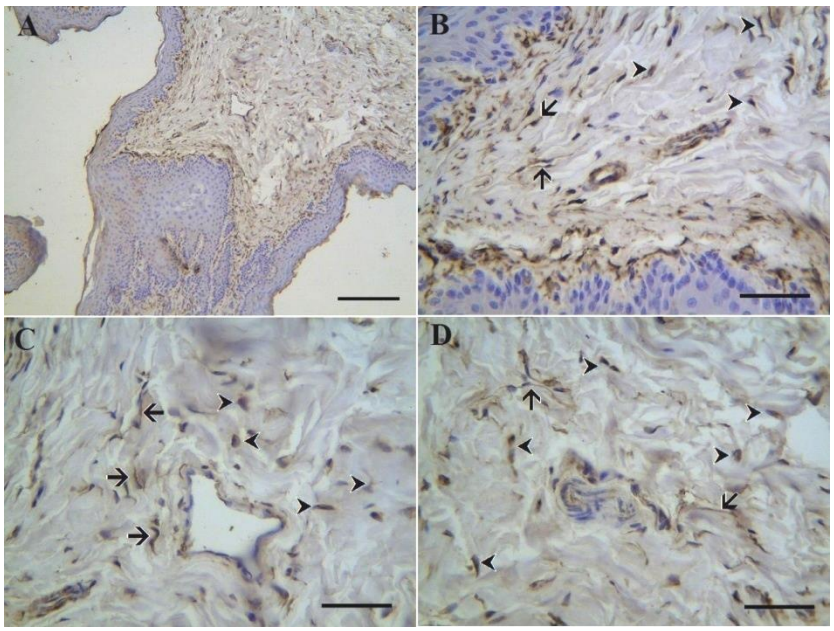

Fig.2: Photomicrograph showing the Vim-ir in the lamina propria. (A) Vim-ir are widely distributed in lamina propria. (B, C, D) A higher magnification view of Fig. A shows the Vim-ir in the fibroblasts (short arrow) or fibroblasts-like cells (longer and thinner processes) (long arrow). Scale bars: A: 200µm; B, C, D: 50µm.

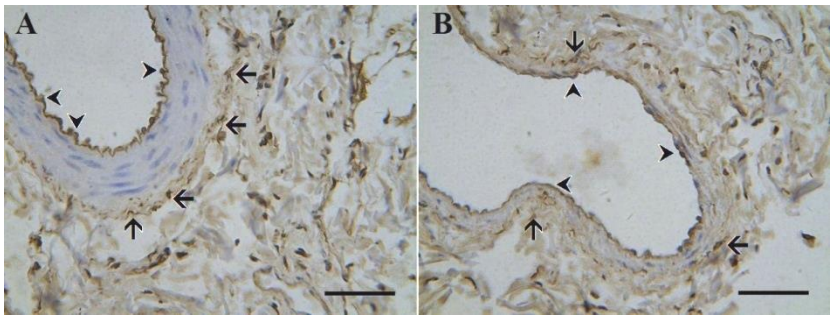

Fig.3: Photomicrograph showing the Vim-ir are labeled in the endothelial cells (short arrow) of arteries (A) and veins (B) and widely distributed around smooth muscles of vessels (long arrow). Scale bars: A, B: 50µm.

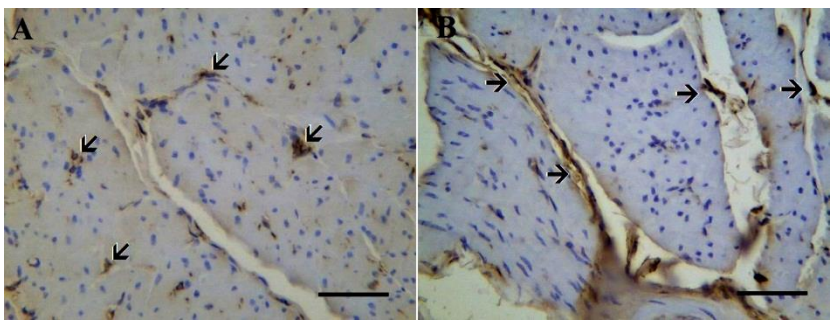

Fig. 4: Photomicrograph showing the Vim-ir in the muscular layer. (A) Vim-ir are strongly stained in the cells of interstitial space between the smooth muscle bundles and these cells show satellite-shaped with some thin processes (long arrow). (B) Inter-communicating processes of positive cells (long arrow) surround fascicles of SMCs, forming a dense reticular network with a particular spatial arrangement. Scale bars: A, B: 50µm.

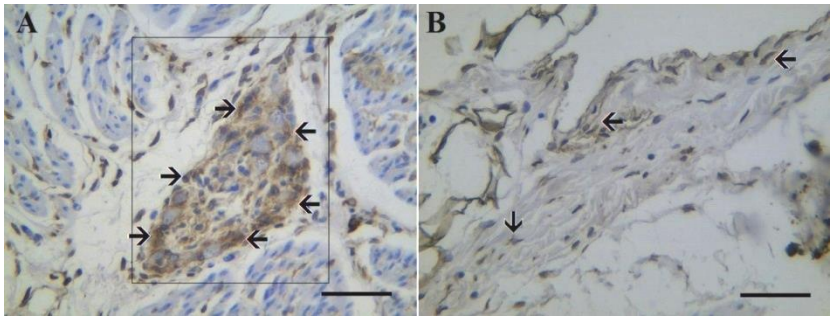

Fig.5: Photomicrograph showing the Vim-ir is present in glia cells (long arrow) of ganglion (square area) (A) and the subserosa (long arrow) (B). Scale bars: A,B: 50µm.

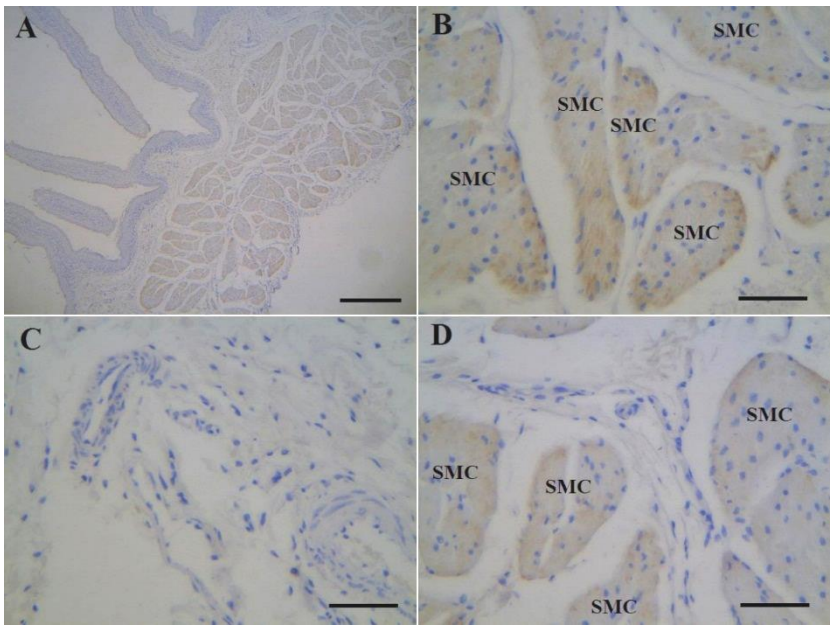

Fig.6: Photomicrograph showing the Des-ir in rumen. (A) Des-ir was dense only in the smooth muscle cells. A higher magnification view of Fig. A shows the Des-ir is present in the smooth muscle cells (SMC) (B) and absent in lamina propria (C) and the interstitial space between the smooth muscle bundles (D). Scale bars: A,B,C,D: 50µm.

Currently, some reports revealed a novel interstitial cell, named telocyte, have extremely long process and can established homo-or heterogenous junctions with neighboring cells to form 3D network. According to their morphological characteristics and distribution, we speculate that all or part of these positive cells is telocytes (TCs). Cretoiu and Popescu suggested that TCs, formerly name as a interstitial-like (Cajal-like) cells (Cretoiu and Popescu, 2014), was coined to prevent further confusion with other interstitial/stromal cells and has been adopted by many laboratories. TCs are novel interstitial cells having specific long prolongations (telopodes) contained alternating lender segments (podomers) with a large dilatations (podoms). Each TC is distinguishable from other interstitial/stromal cells by the characteristic of a small cell body with 2–5 telopodes. Additionally, TCs are also immunohistochemical positive for vimentin. At this point it more confirms our hypothesis. TCs have a close structural connection with other cell types, such as immune cells and stem cells. Most noteworthy, TCs have a direct relationship with smooth muscle cells within the organs as well as in blood vessels and mediate relationship with epithelial cells (Vannucchi and Traini, 2016). Telopodes can form a 3D network structure with complex hetero- or homo-cellular junctions depending on alternation of podom and podomere and their dichotomous branching (Cretoiu and Popescu, 2014). These characteristics of TCs indicate that it have a potential role in the immune response, motility, digestion and absorption of rumen. At present, future mechanism studies are required to clarify the defined role of TCs in rumen.

Along with our results, Vim-ir was also present in the dentritic-like cells, and fibroblasts or fibroblasts-like cells in the subepithelial region, but we did not found Vim-ir localization in the epithelial cells. Whereas, some reports shown that vimentin can be expressed in the M cells and cup cells of the intestine (Fujimura and Iida, 2001; Miller *et al*., 2007). In our results, vim-ir was found in the subepithelial dentritic cells and their processes seem to extend into the epithelial tissue. These findings are in agreement with the previously reported in the human epidermis (Mahrle *et al*., 1983).

Vimentin could be expressed in the glial cells at the different developmental stages. It has been found that the glial cells can be regulate the growth and differentiation of neurons and participate in its connection with the effector cells, promote and inhibit the growth of axons (Arochena *et al*., 2004). Our results shown that the Vim-ir is present in glia cells of ganglion and nerves predominantly in the endoneurium and around perineurium. These findings were similar with the previous report. Our results suggest that the vimentin may be directly involved in the development of the enteric nervous system of rumen, while, still need to explore the further role of vimentin in certain developmental stages and mechanism of interaction between glia cells and neurons within the rumen.

Vim-ir in the muscular layer was widely distributed in the cells with some processes, such as fibroblasts (Köster *et al*., 2015). However, vimentin is a cytoskeletal intermediate filament protein found in the gastrointestinal tract in the submucosa and muscular layer, it can coexist with another marker of c-kit protein in the interstitial cell

of Cajal (ICC) of the gastrointestinal tract (Huizinga *et al*., 2013; Vannucchi and Traini, 2016). But c-kit is a membrane-bound protein present on the cell membrane, so long protuberance, positive reaction display multiple branches between cells and each other to form clear cellular network. While, vimentin is an intermediate filament protein expression was mainly limited to the proximal end of the cell bodies and processes, the finer projections may have intermediate filament protein, but the cell processes with relatively short or small branches, so unable to express well. ICC has the ability to initiate the slow wave activity, thus generating pacemaker activity throughout the gastrointestinal tract. The characteristic of ICC is integral to the coordination of gastrointestinal motility. In addition, ICC is involved in facilitating active propagation of electrical events and mediating neurotransmission (Huizinga *et al*., 2013; Vannucchi and Traini, 2016).

Our results on the distribution of Des-ir in rumen of goat were not similar with the published reports (Vos *et al*., 1993; Marbini *et al*., 1996; Capetanaki *et al*., 2015). For example, those reports showed that desmin was clearly expressed in endothelial cells of blood vessels in the various organs and in the smooth muscle cell bundles of arrectores pilorum muscle of skin (Marbini *et al*., 1996). However, our results showed that the Des-ir was negative in the blood vessels of rumen as compared to the Vim-ir results.

Conclusions: Vimentin and desmin, immunoreactives were not significantly different among various regions of rumen of goat, agreeing mainly with previous reports. The existence of Vim-ir in the special interstitial cells needs to be investigated in detail.

Authors contribution: WL, PY and QC conceived and designed the experiment. WL, PY, NA, JAG and FW performed the experiment and analyzed the data. WL and PY wrote the paper. All authors have contributed to, read and approved the manuscript.

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