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Characteristic abnormal expression of galectin-3 in serrated colon lesions and its pathological significance

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Summary. Serrated lesions are precursors of some colon cancers. The expression of galectin-3 has been reported to be involved in *BRAF* and *KRAS* mutations (the key pathogenic drivers of serrated lesions). This study aimed to investigate the expression intensity and subcellular localization of galectin-3 in serrated colon lesions by immunohistochemistry. The results demonstrated that, regarding expression intensity, galectin-3 expression in serrated colon lesions was significantly upregulated; regarding subcellular localization, the membrane expression of hyperplastic polyps/ sessile serrated lesions (HP/SSL) was weakened, the structure was disorganized and that of traditional serrated adenoma (TSA) was significantly weakened or disappeared, and the nuclear expression of both was positive; in the dysplasia of SSL (SSL-D) and TSA (TSA-HD), galectin-3 expression intensity remained high, and was weakened or disappeared in some nuclei, the expression disorder of the SSL-D cell membrane was reduced, the polarity of the cell was restored, weak expression appeared in the local cell membrane of TSA-HD, and the "serrated" structure of both was reduced or disappeared and seemed to revert more to that seen in common adenomas. In summary, abnormal galectin-3 expression occurs in the early stages of serrated lesions, its expression is characteristic, the dynamic changes in galectin-3 expression are closely related to the histopathological changes and progression of serrated lesions, and further accumulated molecular alterations contribute to this process.

Key words: Serrated colon lesion, Galectin-3, Immunohistochemistry, Expression intensity, Subcellular localization, Pathology

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Introduction

Serrated lesions are precursors to approximately 15-30% of colon cancers (Mezzapesa et al., 2022). The fifth edition of the WHO Classification of Digestive System tumors classifies serrated lesions of the colon into hyperplastic polyps (HP), sessile serrated lesions (SSL), and traditional serrated adenoma (TSA). SSL and TSA are important precancerous lesions of colon cancer, and microvesicular HP and SSL may represent successive stages of a continuous pathological entity (Bateman, 2021).

At the molecular level, the serrated lesion is dominated by early *BRAF* mutations and some *KRAS* mutations. BRAF and KRAS are two key oncogenes in the classic RAS/RAF/MEK/MAP mitogen-activated protein kinase (MAPK) signaling pathway (Sacco et al., 2020). In terms of their pathology, differing from the dysplasia of common adenomas, serrated lesions are mainly manifested by the abnormal location of proliferating zones, and cell proliferation is not obviously active, suggesting that the lesions are related to apoptosis inhibition and cell accumulation, resulting in serrated structural morphology (Figueiredo et al., 2021). We believe that this may also be related to the abnormal connections between cells and the cell matrix and cytoskeleton. Considering the above points, we reviewed the literature and found that the abnormal expression of galectin-3 was involved in the aforementioned important factors of serrated lesions (Cardoso et al., 2016; Kim and Chun, 2020; Aureli et al., 2022).

Studies have shown that galectin-3 plays an important role in the MAPK signaling pathway (Cardoso et al., 2016). Galectin-3 is a 29-35 kDa beta-galactoside binding protein located at the q21-q22 locus of chromosome 14 and is encoded by the *LGALS3* gene. Galectin-3 contains a conserved sequence element of the carbohydrate recognition domain (CRD), which specifically binds β-galactoside, and an N-terminal

domain to form oligomers. Galectin-3 is a multi-functional molecule with different functions according to cell localization: cytoplasmic and nuclear galectin-3 can maintain cell survival by blocking the apoptosis pathway and support the ability of transcription factors to regulate the expression of target genes (such as *Wnt*, *Ras*, *MEK*, and *Notch*). Galectin-3 mediates cell adhesion and separation between cell membranes or cells (Kim and Chun, 2020).

In this study, specimens of normal colonic mucosa, HP, SSL, SSL with dysplasia (SSL-D), TSA, TSA with high-grade dysplasia (TSA-HD), and common adenoma were collected. The expression of the galectin-3 protein was detected by immunohistochemistry, and the characteristics of galectin-3 expression were observed to explore its possible role in the occurrence and development of intestinal serrated lesions to further their understanding and provide evidence for clinical diagnosis and treatment.

Materials and methods

Material collection

Normal colonic mucosa, common adenoma, microvesicular HP, SSL, TSA, SSL-D, and TSA-HD specimens (30, 30, 60, 54, 23, 9, and 5 cases, respectively) were collected from the Department of Pathology, Affiliated Wuxi Hospital of Nanjing Medical University from January 2022 to December 2022. MLH1 expression was lost in two of the nine SSL-D cases, showing microsatellite instability. The pathological diagnosis was strictly based on pathological criteria for the classification of digestive system tumors in the 5th edition of the WHO (Bateman et al., 2021). This study was reviewed by the Ethics Committee of the Affiliated Wuxi People's Hospital of Nanjing Medical University [Nan Yi Da Lun Shen (2019) No. 429].

Immunohistochemical staining

Using the EnVision method, antibodies against galectin-3 (clone number UMAB157) and MLH1 (clone number ES05) were used. All antibodies against galectin-3 were purchased from Beijing Zhongshan Jinqiao Biological Company. PBS was used as a negative control instead of primary antibody. The expression results were analyzed by a double-blind method.

According to the literature (van den Brûle et al., 2000), the expression intensity of galectin-3 was evaluated by the IP score: I was used to evaluate the staining intensity of epithelial cells (0: no staining; 1: brownish-yellow light staining; 2: medium-brown staining; 3: strong dark-brown or tan staining), P is the percentage of epithelial cells assessed with positive staining (0: no staining; 1: 1~10%; 2: 11~33%; 3: 34~66%; 4: 67~100%). The IP score is a semi-quantitative score of intensity x percentage and values range from 0 to 12. Subcellular localization evaluation: that is, the position of galectin-3 expression in cells was interpreted, including 1- cell membrane expression, which was divided into cell membrane regular polar staining, cell membrane expression disorder (manifested as strong, weak and absent, distorted and irregular, polar disturbance, partial expression deletion), and no or significantly weak cell membrane expression; 2- cytoplasmic expression, which can be divided into cytoplasmic expression, no cytoplasmic expression or obviously weak; and 3- nuclear expression, specifically divided into nuclear expression, no nuclear expression, or significantly weak expression. The stained sections were evaluated by two senior pathologists. When there was a disagreement, they reviewed the sections together and discussed to reach an agreement.

Statistical analysis

SPSS 26.0 statistical software and the nonparametric Mann-Whitney test were used to compare the data of independent samples. $P<0.05$ was considered statistically significant.

Results

The expression intensity of the galectin-3 protein in serrated colon lesions was significantly upregulated

After semiquantitative statistical analysis, compared with that of normal intestinal mucosa (Fig. 1A), galectin-3 protein expression in common adenomas (Fig. 1B) and serrated lesions (Fig. 2A for TSA, Fig. 2B for HP, Fig. 2C for SSL) was significantly upregulated ($P<0.05$). The expression of galectin-3 protein in SSL was significantly

Table 1. The expression intensity of the galectin-3 protein in serrated colon lesions.

Expression intensity	I score	P score	IP score	*P* value			
Normal mucosa	1.3±0.48	2.6±0.94	3.6±2.08				
Common adenoma	2.2±0.41	3.7±0.45	8.3±2.08	0.000*			
TSA	2.2±0.42	3.7±0.47	8.3±2.20	0.000*	0.906**		
HP	2.2±0.40	4.0±0.18	8.7±1.69	0.000*	0.078**	0.085***	
SSL	2.4±0.54	4.0±0.14	9.7±2.20	0.000*	0.002**	0.004***	0.006****

*Compared with normal mucosa; **Compared with common adenoma; ***Compared with TSA; ****Compared with HP.

higher than that in common adenomas, TSA, and HP (P=0.002, 0.004, and 0.006, respectively) (Table 1). These data suggest that galectin-3 protein expression may be dynamically increased during the progression from HP to SSL. This finding also suggests that galectin-3 protein expression in HP/SSL may be different from that in common adenomas.

The subcellular localization of galectin-3 expression in serrated colon lesions is characteristic

Compared with that in normal colon mucosa, the subcellular location of galectin-3 expression in common adenomas showed no significant changes, almost all showed cytoplasmic expression, and the cell membrane showed very regular expression, without obvious nuclear expression. However, the cell membrane and nucleus of serrated colon lesions showed obvious characteristic changes. Both HP and SSL (Fig. 2B,C) showed disordered cell membrane expression. Positive expression was found in the nucleus. TSA (Fig. 2A) showed no or weak expression in the membrane and positive expression in the nucleus (Table 2).

Changes in galectin-3 expression intensity and subcellular localization in SSL-D and TSA-HD

The cytoplasmic expression of galectin-3 in SSL and TSA patients with dysplasia continued to be highly expressed, the expression in some SSL-D nuclei (Fig. 3A) was weakened or disappeared, the disturbance of cell membrane expression was reduced, and the polarity was restored (there was no significant difference between SSL-D-microsatellite unstable and SSL-D-microsatellite stable types). The “serrated” structure also decreased or disappeared. Some of the nuclear expression of TSA-HD (Fig. 3B) was also weakened or disappeared, while weak expression appeared in the local cell membrane, and the "serrated" structure was also reduced or disappeared (Table 3).

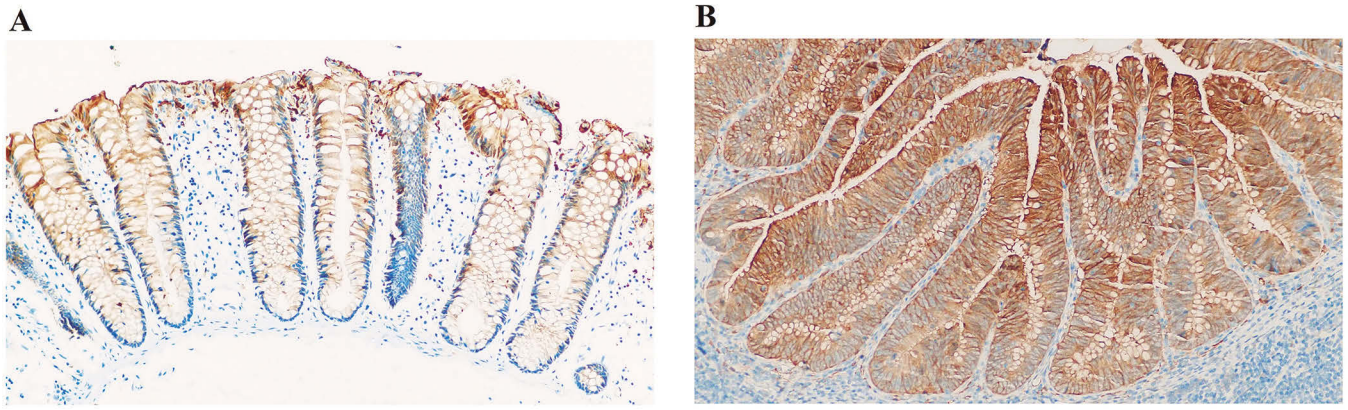

Fig. 1. A. Normal colonic mucosa showing negative or weak galectin-3 expression. **B.** Common adenoma showing high galectin-3 expression and the cell membrane with regular expression, without obvious nuclear expression. Immunohistochemical stain. x 20.

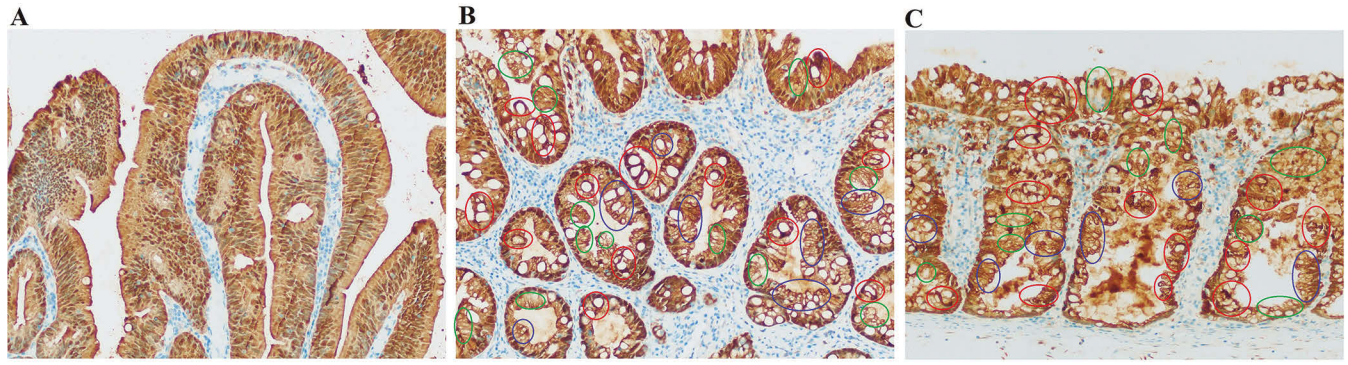

Fig. 2. A. TSA showing high galectin-3 expression, no or weak expression in the membrane, and positive expression in the nucleus. **B, C.** Colon HP and SSL showing high galectin-3 expression, disordered in the cell membrane (manifested as strong (red circle), moderate (blue circle), and no/weak (green circle), distorted and irregular, polar disturbance, partial expression deletion), and positive expression in the nucleus. B was HP, and C was SSL. Immunohistochemical stain. x 20.

Discussion

The occurrence and development of colon cancer mainly involve chromosomal instability (CIN), microsatellite instability (MSI), and the serrated route. *CIN* and *APC* gene mutations are associated with the molecular characteristics of common adenomas. Serrated lesions are primarily characterized by early *BRAF* or *KRAS* mutations (Kim and Bodmer, 2022). *BRAF* and *KRAS* are two key oncogenes in the classic MAPK signaling pathway (RAS-RAF-MEK-ERK pathway) (Sacco et al., 2020). *BRAF* and *KRAS* mutations are usually mutually exclusive, and aberrant activation of both mutations can lead to abnormal activation of the MAPK signaling pathway (Michl et al., 2021).

It was found that galectin-3 selectively interacts with activated K-Ras (K-RAS-GTP) through its CRD and stabilizes it in an "on" state. Activated K-Ras enhances galectin-3 transportation, and galectin-3 activates and controls the strength and duration of K-Ras signaling by promoting the activation of phosphoinositol 3-kinase. High expression of galectin-3, together with K-Ras activation, promotes cell proliferation and survival, leading to tumor formation (Cardoso et al., 2016; Kim

Table 2. Subcellular localization of Galectin-3 expression in serrated colon lesions (n/%).

		Cell membrane expression		Cytoplasmic expression		Nuclear expression	
	Polar regular staining	Expression disorder	No or obviously weak	Yes	No or obviously weak	Yes	No or obviously weak
Normal mucosa (n=30)	27 (90%)	0 (0%)	3 (10%)	27 (90%)	3 (10%)	0 (0%)	30 (100%)
Common adenoma (n=30)	27 (90%)	2 (6.67%)	1 (3.33%)	30 (100%)	0 (0%)	2 (6.67%)	28 (93.33%)
HP (n=60)	0 (0%)	60 (100%)	0 (0%)	60 (100%)	0 (0%)	60 (100%)	0 (0%)
TSA (n=23)	0 (0%)	2 (8.70%)	21 (91.30%)	23 (100%)	0 (0%)	23 (100%)	0 (0%)
SSL (n=54)	0 (0%)	54 (100%)	0 (0%)	54 (100%)	(0%)	54 (100%)	0 (0%)

Table 3. Changes in galectin-3 expression intensity and subcellular localization in SSL-D and TSA-HD.

	Expression intensity	Subcellular localization
SSL-D-microsatellite unstable type (n=2) SSL-D-microsatellite stable type (n=7)	continued to be highly expressed	the expression in some SSL-D nuclei was weakened or disappeared, the disturbance of cell membrane expression was reduced, and the polarity was restored. The “serrated” structure decreased or disappeared.
TSA-D (n=5)	continued to be highly expressed	Some of the nuclear expression was weakened or disappeared, while weak expression appeared in the local cell membrane, and the "serrated" structure was also reduced or disappeared.

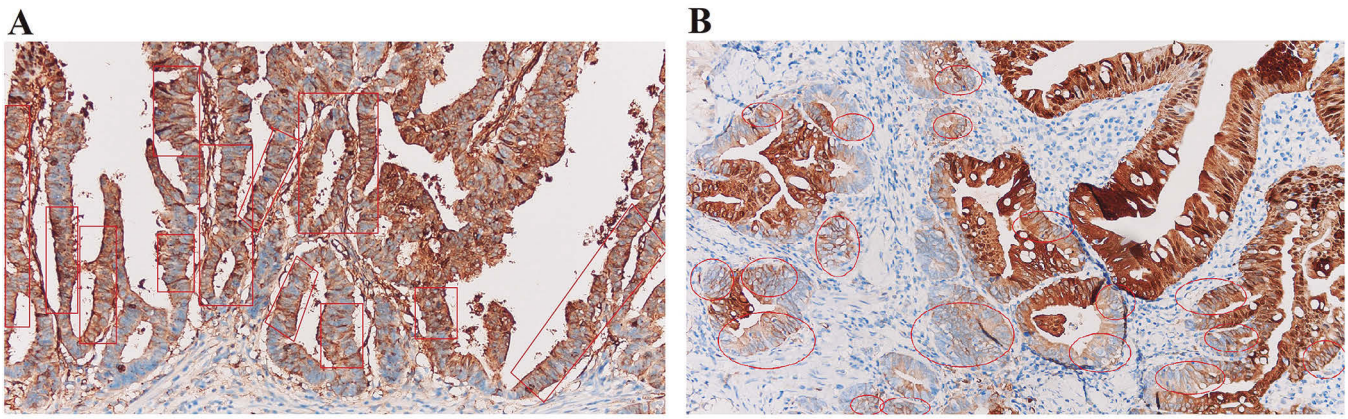

Fig. 3. A. The expression of galectin-3 in SSL-D continued to be highly expressed, the expression of some SSL-D nuclei was weakened or disappeared, the disturbance of cell membrane expression was reduced, and the polarity was restored (red square). The “serrated” structure decreased or disappeared. **B.** The expression of galectin-3 in TSA-HD continued to be highly expressed, the nuclear expression of TSA-HD was weakened or disappeared, while weak expression appeared in the local cell membrane (red circle), and the "serrated" structure was also reduced or disappeared.

and Chun, 2020). The results of this study show that galectin-3 was characterized by abnormal expression of galectin-3 in the early stages of serrated colon lesions, including changes in expression intensity and subcellular localization. This suggests that galectin-3 may be involved in the histopathological formation of serrated colon lesions and may be related to the abnormal activation of MAPK signaling. According to the different abnormal expression results of galectin-3 in HP/SSL and TSA in this study, we speculated that the specific molecular mechanism and effect of *BRAF* and *KRAS* mutations on galectin-3 may be different in serrated lesions, deserving further study.

Galectin-3 is a cytoplasmically synthesized protein that can shuttle between the cytoplasm and nucleus, can be transported to the membrane, and even secreted into the extracellular environment, whose role depends on its subcellular localization (Kim and Chun, 2020). Here, we attempted to describe the relationship between the observed changes in galectin-3 expression in serrated lesions and known molecular pathology, mainly in terms of apoptosis inhibition and abnormalities in the intercellular and cell-matrix connections and skeleton, as previously mentioned. The details are as follows:

Apoptosis inhibition: Cytoplasmic and nuclear galectin-3 are involved in many types of anti-apoptotic processes. Galectin-3 has significant sequence homology with the anti-apoptotic protein Bcl2 (48% protein sequence similarity), and its CRD region has a four-amino acid motif, namely, Asn-Trp-Gly-Arg (NWGR motif) (amino acid residues 180-183). This motif is highly conserved in the BH1 domain (amino acid residues 143-146) of the Bcl-2 family (Kim and Chun, 2020). Moreover, galectin-3 is the only member of the beta-galactoside binding protein family that contains this NWGR motif, and galectin-3 and Bcl2 proteins can play an anti-apoptotic role by forming heterodimers (Jeethy Ram et al., 2021). Galectin-3 is also closely related to cyclins (cyclin D1, E, and A) and the inhibitory proteins p16, p21, and p27, which play a role in inhibiting apoptosis (Jeethy Ram et al., 2021).

Abnormalities in the intercellular and cell-matrix connections: This study showed that galectin-3 membrane expression was characteristic of serrated colon lesions. Interestingly, with the reduction in galectin-3 expression disorder in the SSL-D cell membrane and the appearance of galectin-3 expression in the TSA-HD cell membrane, the "serrated" structure also decreased or disappeared. It is inferred that abnormal expression of galectin-3 in the cell membrane may play a unique role in the formation of the serrated pathological morphology. Currently, many studies reported that galectin-3 on the surface of the cell membrane or between cells can mediate cell adhesion and separation by cell adhesion molecules (Xin et al., 2015; Kim and Chun, 2020). Galectin-3 can bind to β-catenin and mediate the expression and function of E-cadherin by regulating and activating the Wnt/β-catenin signaling pathway. Downregulation of E-cadherin expression can lead to decreased intercellular adhesion strength and damaged cell arrangement. Galectin-3 can also regulate the adhesion and migration of tumor cells by regulating integrins to the extracellular matrix, promoting the aggregation of integrins on the cell surface, mediating the endocytosis of integrins, or directly activating integrins.

It is very interesting that when serrated lesions become dysplastic, the pattern of galectin-3 expression reverts more to that seen in common adenomas. Our results showed that galectin-3 expression was weakened or disappeared in some nuclei during the development of SSL and TSA, similar to the translocation of galectin-3 from the nucleus to the cytoplasm in the progression of colon cancer (Wang and Guo, 2016). Studies have shown that galectin-3 can shuttle between the nucleus and cytoplasm through certain mechanisms related to the cell stress response, tumor progression, and other functions (Sacco et al., 2020). Further accumulation of molecular alterations, usually with abnormal WNT signaling during the development of serrated lesions, should be important mechanisms (Mikhaleva et al., 2021; Satorres et al., 2021). It was reported that galectin-3 is a novel binding partner of β-catenin. Both galectin-3 and β-catenin are substrates of casein kinase I and glycogen synthase kinase-3β (Shimura et al., 2005). This suggests that when WNT signaling is activated, it may also act inversely on the expression pattern of galectin-3 as a molecular switch (Shimura et al., 2005), which requires further investigation in serrated colon lesions.

In summary, we hypothesized that early *BRAF* or *KRAS* mutations trigger activation of the classic MAPK signaling pathway in the early or immediate onset of serrated lesions, leading to abnormal expression and functional activation of downstream galectin-3, whose antiapoptotic effect increases the number of cells. The disturbance of intercell and cell-matrix connections will cause the abnormal spatial structure of cell arrangements, which together cause the abnormal accumulation of cells, thus forming "serrated" pathologic changes. The dynamic changes of galectin-3 expression and the progression of serrated lesions may involve further cumulative molecular alterations.

Author contributions. Zhiyi Zhou, Dandan Huang, and Qiang Zhan designed the research, analyzed histopathological data, constructed figures and tables, and drafted the manuscript. Nanxing Jiang performed the immunostaining. Ying Cai and Shudong Yang provided suggestions for the experimental design and manuscript drafting.
Conflict of interest. The authors declare no conflict of interest.

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