K-RAS, B-RAF and GNAS Gene Status and Immunohistochemistry Analysis of Mucinous Neoplasm of Appendix

xinyu ren (renxinyu7956@163.com)

Departments of Pathology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Science, Beijing, China

Yin Cheng

Departments of Pathology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, 100045, China

Tao Lu

Departments of Pathology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Science, Beijing, China

Junliang Lu

Departments of Pathology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Science, Beijing, China

Yan Wu

Departments of Pathology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Science, Beijing, China

Junyi Pang

Departments of Pathology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Science, Beijing, China

Longyun Chen

Departments of Pathology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Science, Beijing, China

Weixun Zhou

Departments of Pathology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Science, Beijing, China

Zhiyong Liang

Departments of Pathology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Science, Beijing, China

---

Research

Keywords: low grade appendiceal mucinous neoplasm, serrated lesions, MUC5AC, MUC1, MUC6, K-ras, b-raf, GNAS

Posted Date: August 14th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-57384/v1

Abstract

AIM

Low grade appendiceal mucinous neoplasm (LAMN) and serrated lesions are sometimes hard to differentiate from morphology. We try to characterize them from the immunohistochemical and molecular perspective and to reflect the difference between them.

METHODS

25 appendix neoplasm including 13 LAMNs, 12 serrated lesions were selected from January 2013 to December 2014. Immunohistochemical analyses for cytokeratin 20, MUC6, MUC5AC, MUC1, Ki-67, P53 and mismatch repair (MMR) proteins including MLH1, PMS-2, MSH-6, MSH-2 were performed. Microsatellite instability (MSI) status was also evaluated. Besides, we detect K-RAS, B-RAF and GNAS gene mutation status of these lesions.

RESULTS

Immunochemically, 83.3% serrated lesions showed scattered CK20 staining in the deep crypt, which was less so for LAMNs. As for mucin expression, MUC5AC had slightly higher positive rate in LAMNs and than in serrated lesions.MUC1 was significantly higher expressed in LAMNs than in serrated lesions. 46.1% LAMNs have P53 expression in deep crypt, while P53 was negative in the deep crypt of serrated lesions. 58.3% serrated lesions had deficient MMR protein expresion pattern compared to 23.1% of LAMNs. B-RAF mutation was detected in 3 cases, all were serrated lesions. K-ras and GNAS mutation was detected in both LAMNs and serrated lesions.

CONCLUSION

Immunohistochemical panel comprising markers such as CK20, MUC5AC, MUC1, Ki-67 and P53, with genotyping covering hotspots of the *KRAS*, *BRAF* and *GNAS* genes can help the differential diagnosis of low grade appendix neoplasm.

Introduction

Low grade appendiceal mucinous tumor (LAMN) and appendiceal serrated lesion are low grade appendix neoplasms that share similar clinical presentations whereas variable biological behaviors, from a benign indolent course to a relatively aggressive malignant process.

LAMN is an appendiceal neoplasm characterized by replacement of the nomal appendiceal mucosa by a filiform villous mucinous epithelial proliferation. Pseudomyxoma peritonei (PMP) that originated from

ruptured mucinous appendiceal tumor will have a low 5- year survival rate[1]. Therefore, it is necessary to differentiate LAMN from the other benign lesions, such as serrated lesion, before it ruptured.

From the authors' experience, LAMN, appendiceal serrated lesion are difficult to differentiate, due to overlaps in the morphology. Several research groups have used immunohistochemical analyses (cytokeratin20, Ki-67, MUC6, and β-catenin) to differentiate different types of serrated polyps [2–4]. Yet whether the panel of CK20, Ki-67, and mucins are useful in differentiating serrated and non-serrated lesions remains poor clarified. It is also established that serrated neoplasm from colon and rectum are precursor lesions for sporadic MSI-H cancers and most sessile serrated adenoma/polyps (SSA/Ps) harbor mutations in *BRAF* (up to 90%) [5, 6].Since serrated polyps in the appendix showed similar morphology to their colorectal counterparts, there are many reports focusing on the genetic alterations in appendix such as *K-ras* ,*BRAF* and *GNAS*, but few focus on dMMR status and relavant molecular changes.

In this study, we compared the immunophenotypic and molecular profiles of different low-grade neoplasms of appendix to better understand the difference between the two lesions.

Materials And Methods

Patients and Diagnosis

We searched the pathology electronic documentations of the Peking Union Medical College Hospital (2013 to 2014) for appendiceal lesions using the following keywords: low grade appendiceal mucinous neoplasm, hyperplastic polyp, sessile serrated adenoma, serrated adenoma, and tubular adenoma. Corresponding H&E slides were retrieved and reviewed by 4 pathologists independently by consensus to the following categories: serrated lesions and LAMN. Serrated lesions included HPs and Serrated desplasia, which were diangosed based on distorted crypts with serration crypt dilatation extending to crypt bases with or without cytologic dysplasia. LAMNs were diagnosed by a filiform villous mucinons type or columnar epithelial cells with nuclear pseudostratification growing on a fibrotic submucosal tissue.

Immunohistochemical analysis

Immunohistochemical analyses were performed on 4-μm sections made from a representative tissue block from each of the cases on Autostainers. The antibody information including clone, vendor, dilution, positive pattern and the proper antigen retrieval condition were listed in Supplementary Table 1. Positive and negative controls were included in each batch of staining. For MMR components (MLH1, PMS-2, MSH-6, MSH-2), tumour stromal cells and inflammatory cells were used as internal controls, loss of MMR protein expression was determined as defective MMR (dMMR).

MSI status analysis

Microsatellite analysis was performed as described previously using Microsatellite Instability Detection Kit (Microread, China) [7]. In brief, DNA was first extracted from paraffin-embedded tissue blocks and then conduct multiplex fluorescent polymerasechain reaction (PCR). MSI locus recommended by NCCN guideline such as NR-21, NR-24, NR-27, BAT-25, BAT-26, Mono-27 was amplified and detected. New PCR peaks appeared in two or more of six locus was regarded as high-frequency MSI (MSI-H), in one of six locus as low-frequency (MSI-L), and no new peaks in six locus as microsatellite stable (MSS).

BRAF, KRAS **and** GNAS **mutation analysis**

Appendiceal lesions were enriched by macro dissection from 4-micron sections. DNA was extracted using the DNA extraction kit (AmoyDx ® FFPE DNA Kit, Cat No. ADx-FF01, Amoy Diagnostics Co, China). *KRAS* and *BRAF* mutation analyses were accomplished using commercially available ARMS-PCR based assays (AmoyDx® *BRAF* 600 and *KRAS* mutations detection kit ADx-BR04-R, ADx-KR05, Amoy Diagnostics Co, China) for 25 cases according to the manufacturer's instructions. The coverage of the kits was detailed in Supplementary Table 2. *GNAS* mutation was analyzed by Sanger sequencing. The primers were designed as follows to amplify DNA fragment including exon 201 of the gene. Forward: AGACCTTTGCTTTAGATTGGC; reverse: CTTACTGGAAGTTGACTTTGTCC. This primer pair yielded a PCR product of 157 bp. The purified fragments were subjected to direct sequencing on the Genetic Sequencer ABI3500 (Applied Biosystems, Grand Island, USA) using the BigDye® Direct Sanger Sequencing Kit following the manufacturer’s protocol.

Statistical analysis

Statistical analysis was performed by SPSS 17.0 (SPSS, Chicago, IL, USA).Differences in immunohistochemical indexs and gene mutations between groups were assessed with $\chi 2$ test. All statistics were assessed using two-sided tests with a $P < 0.05$ being considered statistically significant.

Results

LAMN presented female predilection [8], the incidence ratio of female to male is 11/2. The average ages for the two groups were 63.5 and 66.7years, respectively. One case of LAMN was coincidentally found in colon carcinoma resection. 6 out of 13 LAMNs had coexisting PMP. For serrated lesions, only one was found by appendectomy due to acute appendicitis. The patient charateristics were summarized in Table 1.

Table 1
Clinicopathologic features of patients with appendiceal lesions

Clinicopathologic features	LAMN	Serrated lesions
Total no. of cases	13	12
Gender(F/M)	11/2	7/5
Average age	63.5	66.7
Lesion size (mm)(range)	2 × 1 × 1 cm-4.2 × 1.5 × 1.5 cm	0.3 cm-4.7 × 0.6 × 0.6 cm
Gross cystic dilatation	8	0
Gross luminal mucin	13	12
Gross rupture	4	0
Polyp growth pattern	1	2
Discrete polyp	1	2
Associated lesions		
Colon carcinoma	3	4
IBD (UC or CD)	0	0
Overy carcinoma	0	3
PMP	9	0
Appendicitis	1	1

IBD: inflammatory bowl disease; UC: ulcerative colitis; CD: Crohn's disease; PMP:pseudomyxoma peritonei

Immunohistochemically, CK20 was expressed in the surface epithelium in two types of lesions. But in the deep crypts, CK20 was positive in 10 of 12 serrated lesions and 3 out of 12 LAMNs (Fig. 1). The difference was statistically significant(Table 2). Five and six LAMNs and serrated lesions showed focal or scatter positivity for MUC-6, respectively. All LAMN cases and 11 of 12 cases of serrated lesions were MUC5AC positive. Regarding to MUC1, 12 out of 13 LAMNs were positive, at the mean time 4 serrated lesions were immunoreactive. None of the serrated lesions were positive for P53, while 6 LAMNs were P53 positive. In the two entities, Ki-67 were mostly located in the deep crypts, while in four LAMNs and one serrated lesions, the staining went to the upper part of the crypt.

Table 2
Immunohistochemical features of study patients with appendiceal lesions

Immunohistochemical features	LAMN	Serrated lesions	P Value
Total no. of cases	13	12	
CK20	Diffuse positive, 3 with crypt scatter positive	Diffuse positive, 10 with crypt scatter positive	0.005*
MUC6	5 focal positive	6 focal or scatter positive	0.695
MUC5AC	13 diffuse positive	11 diffuse positive	0.458
MUC1	12 focal surface epithelium positive	4 focal surface epithelium positive	0.008*
P53	6 positive	0 positive	0.015*
Ki-67	4 case positive both in the crypt and in surface epithelium, 9 with crypt positive	1 case irregular positive, 11 cases with crypt positive	0.322
P Vaue was calculated using $\chi 2$ test.			
* statistically significant different			

In terms of the four mismatch repair proteins, MLH1, PMS2, MSH2 and MSH6, LAMN, serrated lesions both had dMMR. The difference was statistically significant (P = 0.047). Immunohistochemical interpretations of all markers were summarized in Table 3. Three of thirteen LAMN have loss of MSH6 in the villous structure, in serrated lesions, 7 of 12 serrated lesions have dMMR in the protein level including loss of MSH6, PMS2 and MLH1, although direct analyses of representative MSI loci showed none of serrated lesions of the appendix were MSI-H(Table 3). Results of mutational analyses were listed in Table 4. Four types of K-ras mutation were detected, including G12D, G12V, G13D and G12C. *KRAS* mutation were detected in 12 LAMNs and and 5 serrated lesions. Among them, G12D was the most common genotype, distributing in eight LAMNs and two serrated lesions. Of note, the only one G12C mutation was occurred in a serrated lesion. The difference in the frequencies of *KRAS* mutation in the two groups was statistically significant (P = 0.011). Only serrated lesion carried *BRAF* mutation in the present case series (3/12). *GNAS* mutation was found in 5 LAMN cases, 2 serrated lesions, respectively. The difference in *GNAS* mutation profile among groups was not significant (P = 0.378). Interestingly, all of *GNAS* mutation (8 of 8 cases) in both lesions was found to coexist with *KRAS* mutation. (Fig. 2G).

Table 3
Immunohistochemical and molecular Micro-satellite state

	LAMN	Serrated lesions	P Value
Total case	13	12	
dMMR	3	7	0.047*
PMS2	All preserved	8 preserved, 4 deficient	
MHL1	All preserved	3 deficient	
MSH6	3 deficient	8 deficient	
MSH2	All preserved	All preserved	
MSI-DNA			1.000*
MSS	12	11	
MSI-H	0	0	
MSI-L	1	1	
P Vaue was calculated using $\chi 2$ test.			
* statistically significant different			

Table 4
Molecular features of study patients with appendiceal lesions

molecular features	LAMN	Serrated lesions	P Value
Total case	13	12	
K-RAS(Cases /Mutation type)	12	5	0.011*
G12V/35G > T	2(1case co-exist GNAS mutation)	0	
G12D/35G > A	8(3cases co-exist GNAS mutation)	2(1 case co-exist GNAS mutation)	
G13D/38G > A	2(1 case co-exist GNAS mutation)	2(1 case co-exist GNAS mutation)	
G12C/34G > T	0	1	
No mutaion	1	7	
B-RAF	0	3(with no other mutation)	0.096
G-NAS(Cases /Mutation type)	5	2	0.378
602G□A	2	2	
601C□T	3	0	
No mutation	8	10	
P Vaue was calculated using χ2 test.			
* statistically significant different			

Discussion

LAMN, and serrated lesion are two major appendiceal premalignant lesions, they morphologically resemble each other. Sometimes, when the lesion is small and is not as typical, it is difficult to distinguish them. We try to look for their differences from the immunohistochemical and genetic point of view.

Torlakovic and his colleagues used CK20 and Ki-67 immunohistochemistry to appreciated the aberrant maturation and proliferation of the crypts in serrated lesions[2]. In a large proportion of serrated lesions in our study, scattered CK20 positivity in the deep crypt was observed, which wasn't different from Torlakovic's findings. This phenomenon was also observed by Andrew M. Bellizzi et al[9]. Of note, a significantly smaller fraction of LAMN also showed this staining pattern, which has not been previously reported. So, CK20 positivity in the deep crypt was charatrastic of serrated lesions in appendix.

In the present study, 6 in 13 of LAMN cases showed P53 scattered positive in deep crypt.This rate was relatively higher than results of Hara et all[10], Besides, all the P53 positive LAMNs patients were female and had concomitant PMP, which was in concordance with existing literatures, P53 over-expression in PMP of appendiceal origin was significantly related to female sex, spreading to the abdominal cavity, and worse survival for patient[11].In serrated lesions, P53 were all negative, even in cases with epithelial dysplasia, this result was the same as Yantiss et all[12], only 1 serrated adenoma showed more than 10% of the surface epithelium positive. This may imply that serrated lesions in appendix was mostly low grade, the reason might be they lack of mucus secretion and lower p53 expression.

Ki-67 was mostly expressed in deep crypt of LAMN (9/13) and serrated lesions (11/12), while four LAMNs and one serrated lesions have Ki-67 staining in surface epithelium as in conventional colon adenomas.

Different types of mucins expression with varying strength has been reported in serrated lesions and adenomas of the colon and rectum [13–15]. We therefore chose three types of mucins, including MUC6, MUC5AC, and MUC1. MUC6 was already showed to associate with morphologic serrated features of appendix in the study of Bellizzi et al [9]. Our result showed that LAMN and serrated lesions both can have MUC6 expression (5/13, 6/12), although only in focal area.

LAMN shared similarity with pancreatobiliary subtype of pancreatic intraductal papillary mucinous neoplasm (IPMN), which is usually positive for MUC5AC and MUC1 and negatively for MUC6 [16, 17]. MUC5AC and MUC1 were indeed highly expressed in LAMN in our cases. Besides, serrated lesions also have high MUC5AC expression and 6 of 12 serrated lesions have focal MUC6 expression as LAMN. This indicated that both LAMN and serrated lesions of appendix can have MUC5AC and focal MUC6 expression, and a combination of MUC5AC and MUC1 might help us to distinguish LAMN from other lesions of the appendix. The possible mechanism may be *GNAS* mutation, which was characteristic of LAMN[18], could induce MUC5AC and MUC2 expression in colorectal cancer cell lines, so in LAMN, MUC5AC is all positive in both crypt and surface epithelium. Focal MUC6 expression in both LAMN and serrated lesions may explain their morphologic similarities. Besides, in Mesa's research[19], MMR deficiency in appendix neoplasms showed a correlation with MUC5AC and MUC6 expression. In our LAMN and serrated cases, 11/24 that have MUC5AC expression also have dMMR, and 6/11 that have focal MUC6 expression also have dMMR.

Serrated lesions of colon carcinoma tend to have higher rate of microsatellite instability[20]. In concordance with colon carcinoma, our result also showed that serrated lesions in appendix are prone to have dMMR than LAMN.7 of 12 serrated lesions have dMMR in the protein level including loss of MSH6, PMS2 and MLH1, although Direct analyses of representative MSI loci showed none of serrated lesions of the appendix were MSI-H. Yantiss et all [12]also reported that incomplete loss of MLH1 protein in serrated polyps of appendix was not accompanied by MSI-H. In our cases, three LAMNs have loss of MSH6 in the villous structure but no loss of MSH2, although this type was rarely reported in LAMN, only in MSI-high

appendiceal carcinoma[21]. This indicated that LAMN can also have microsatellite instability, even though they are at an early stage.

KRAS mutation in LAMN was significantly higher than in serrated lesions, with 12 of 13 cases having different type of mutations. This is consisted with the previous reports[18, 12, 22, 23]. In their study, 94% cases of LAMN were *KRAS* mutated and the most frequent mutation type was 35G > A. 5 of 12 serrated lesions also have K-ras mutations, which indicated that LAMN and serrated lesions may share similar oncogenic pathways.

Several articles reported a considerable proportion of LAMNs harboring *GNAS* mutation[24, 18]. In our study, only 5 of 13 (38.5%) LAMN cases exists *GNAS* mutation. This rate was higher than the serrated lesions. This relatively low rate of *GNAS* mutation in LAMNs might be due to the low sensitivity of Sanger sequencing and limited exon coverage. Alakus and other researchers [24–27] also proposed that co-existing mutations of *KRAS* and *GNAS* were characteristic to LAMN and *KRAS* mutation occurs earlier in the course of tumorigenesis. This pathway was also shared by IPMN. In the present study, 8 cases, including both LAMN and serrated lesions, that have *GNAS* mutation, also have *KRAS* mutation co-exist. These may have indicated that *GNAS* mutation was prone to happen following the *KRAS* mutation.

BRAF mutation was only presented in 3/12 cases of serrated lesion. Rish K. Pai et al [28] found serrated lesions in appendix harbor more *KRAS* mutations instead of *BRAF* mutations, by which they regarded serrated lesions in appendix as a distinct entity from their counterparts in the colon, which was supposed to have more *BRAF* mutation.Other researchers[25] reported higher rate of *BRAF* mutation of the serrated lesion. Our result also indicated a slightly more *KRAS* mutation (5/12) in serrated lesions than *BRAF* mutation (3/12) in the appendix and our histological critieria was almost the same with them. *BRAF* mutation was only confined to serrated lesions in the present study, suggestting that *BRAF* could be used as a specific yet insensitive marker for serrated lesion in appendix. Similar as the colorectal counterparts, serrated lesion in appendix can have dMMR and *BRAF* mutation.

In conclusion, LAMN and serrated lesion each had their characteristic immunohistochemical expression and molecular mutations, LAMN was both MUC1 and MUC5AC positive and harbors both *KRAS* and *GNAS* mutation, and they can also have loss of MSH6 immunoexpression and also have high Ki67 index in the surface epithelium. Serrated lesions can also have dMMR, with MUC5AC and MUC6 focal expression and they were all p53 negative and they also harbor *KRAS* mutation. Combination of these different markers could asist the differentiation.

Abbreviations

LAMN: Low grade appendiceal mucinous neoplasm; MMR: mismatch repair; PMP: Pseudomyxoma peritonei; SSA/Ps: sessile serrated adenoma/polyps; dMMR: defective MMR; PCR : polymerasechain reaction; MSI-H : high-frequency MSI; MSI-L : low-frequency MSI; MSS : microsatellite stable

Declarations

Author Contributions Statement

Xinyu Ren, Weixun Zhou and Zhiyong Liang reviewed the case together. Xinyu Ren and Yin Cheng wrote the article. Tao Lu and Yan Wu did the mutation test. JunyiPang did the Immunohistochemical test.

- **Conflict of interest**
- The submission was approved by all authors and none of the authors has any potential financial conflict of interest related to the manuscript.

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study obtained the approval of the ethics committee of Peking Union Medical College Hospital. Written informed consent was obtained from each patient.

Consent for publication

Not applicable. All authors read and approved the final manuscript.

- **Acknowledgments**
- This work was supported by CAMS Initiative for Innovative Medicine (CAMS-I2M) (2016-I2M-1-002) and CAMS Central Public Welfare Scientific Research Institute Basal Research Funds Clinical and Translational Medicine Research Program(2019XK320045).

References

1. Smeenk RM, Verwaal VJ, Antonini N, Zoetmulder FA. Survival analysis of pseudomyxoma peritonei patients treated by cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. Annals of surgery. 2007;245(1):104–9. doi:10.1097/01.sla.0000231705.40081.1a.
2. Torlakovic EE, Gomez JD, Driman DK, Parfitt JR, Wang C, Benerjee T, Snover DC. Sessile serrated adenoma (SSA) vs. traditional serrated adenoma (TSA). Am J Surg Pathol. 2008;32(1):21–9. doi:10.1097/PAS.0b013e318157f002.
3. Owens SR, Chiosea SI, Kuan SF. Selective expression of gastric mucin MUC6 in colonic sessile serrated adenoma but not in hyperplastic polyp aids in morphological diagnosis of serrated polyps. Modern pathology: an official journal of the United States Canadian Academy of Pathology Inc. 2008;21(6):660–9. doi:10.1038/modpathol.2008.55.

4. Wu JM, Montgomery EA, Iacobuzio-Donahue CA. Frequent beta-catenin nuclear labeling in sessile serrated polyps of the colorectum with neoplastic potential. Am J Clin Pathol. 2008;129(3):416–23. doi:10.1309/603UQKM7C2KELGJU.
5. Carr NJ, Mahajan H, Tan KL, Hawkins NJ, Ward RL. Serrated and non-serrated polyps of the colorectum: their prevalence in an unselected case series and correlation of BRAF mutation analysis with the diagnosis of sessile serrated adenoma. J Clin Pathol. 2009;62(6):516–8. doi:10.1136/jcp.2008.061960.
6. Spring KJ, Zhao ZZ, Karamatic R, Walsh MD, Whitehall VL, Pike T, Simms LA, Young J, James M, Montgomery GW, Appleyard M, Hewett D, Togashi K, Jass JR, Leggett BA. High prevalence of sessile serrated adenomas with BRAF mutations: a prospective study of patients undergoing colonoscopy. Gastroenterology. 2006;131(5):1400–7. doi:10.1053/j.gastro.2006.08.038.
7. Du C, Zhao J, Xue W, Dou F, Gu J. Prognostic value of microsatellite instability in sporadic locally advanced rectal cancer following neoadjuvant radiotherapy. Histopathology. 2013;62(5):723–30. doi:10.1111/his.12069.
8. Rymer B, Forsythe RO, Husada G. Mucocoele and mucinous tumours of the appendix: A review of the literature. International journal of surgery. 2015;18:132–5. doi:10.1016/j.ijsu.2015.04.052.
9. Bellizzi AM, Rock J, Marsh WL, Frankel WL. Serrated lesions of the appendix: a morphologic and immunohistochemical appraisal. Am J Clin Pathol. 2010;133(4):623–32. doi:10.1309/AJCP1UJPX6UURLCH.
10. Hara K, Saito T, Hayashi T, Yimit A, Takahashi M, Mitani K, Takahashi M, Yao T. A mutation spectrum that includes GNAS, KRAS and TP53 may be shared by mucinous neoplasms of the appendix. Pathol Res Pract. 2015;211(9):657–64. doi:10.1016/j.prp.2015.06.004.
11. Shetty S, Thomas P, Ramanan B, Sharma P, Govindarajan V, Loggie B. Kras mutations and p53 overexpression in pseudomyxoma peritonei: association with phenotype and prognosis. J Surg Res. 2013;180(1):97–103. doi:10.1016/j.jss.2012.10.053.
12. Yantiss RK, Panczykowski A, Misdraji J, Hahn HP, Odze RD, Rennert H, Chen YT. A comprehensive study of nondysplastic and dysplastic serrated polyps of the vermiform appendix. Am J Surg Pathol. 2007;31(11):1742–53. doi:10.1097/PAS.0b013e31806bee6d.
13. Mochizuka A, Uehara T, Nakamura T, Kobayashi Y, Ota H. Hyperplastic polyps and sessile serrated ‘adenomas’ of the colon and rectum display gastric pyloric differentiation. Histochem Cell Biol. 2007;128(5):445–55. doi:10.1007/s00418-007-0326-2.
14. Biemer-Huttmann AE, Walsh MD, McGuckin MA, Ajioka Y, Watanabe H, Leggett BA, Jass JR. Immunohistochemical staining patterns of MUC1, MUC2, MUC4, and MUC5AC mucins in hyperplastic polyps, serrated adenomas, and traditional adenomas of the colorectum. The journal of histochemistry cytochemistry: official journal of the Histochemistry Society. 1999;47(8):1039–48. doi:10.1177/002215549904700808.
15. Hirono H, Ajioka Y, Watanabe H, Baba Y, Tozawa E, Nishikura K, Mukai G, Honma T, Aoyagi Y. Bidirectional gastric differentiation in cellular mucin phenotype (foveolar and pyloric) in serrated

adenoma and hyperplastic polyp of the colorectum. Pathology international. 2004;54(6):401–7. doi:10.1111/j.1440-1827.2004.01639.x.

16. Moschovis D, Bamias G, Delladetsima I. Mucins in neoplasms of pancreas, ampulla of Vater and biliary system. World J Gastrointest Oncol. 2016;8(10):725–34. doi:10.4251/wjgo.v8.i10.725.
17. Masaki Y, Oka M, Ogura Y, Ueno T, Nishihara K, Tangoku A, Takahashi M, Yamamoto M, Irimura T. Sialylated MUC1 mucin expression in normal pancreas, benign pancreatic lesions, and pancreatic ductal adenocarcinoma. Hepato-gastroenterology. 1999;46(28):2240–5.
18. Nishikawa G, Sekine S, Ogawa R, Matsubara A, Mori T, Taniguchi H, Kushima R, Hiraoka N, Tsuta K, Tsuda H, Kanai Y. Frequent GNAS mutations in low-grade appendiceal mucinous neoplasms. Br J Cancer. 2013;108(4):951–8. doi:10.1038/bjc.2013.47.
19. Mesa H, Manivel JC, Larson WS, Dachel SK, Reinink AR, Jessurun J. Immunophenotypic Comparison of Neoplasms of the Appendix, Right Colon, and Left Colon in Search of a Site-Specific Phenotypic Signature. Int J Surg Pathol. 2020;28(1):20–30. doi:10.1177/1066896919859096.
20. O'Brien MJ, Yang S, Mack C, Xu H, Huang CS, Mulcahy E, Amorosino M, Farraye FA. Comparison of microsatellite instability, CpG island methylation phenotype, BRAF and KRAS status in serrated polyps and traditional adenomas indicates separate pathways to distinct colorectal carcinoma end points. Am J Surg Pathol. 2006;30(12):1491–501. doi:10.1097/01.pas.0000213313.36306.85.
21. Taggart MW, Galbincea J, Mansfield PF, Fournier KF, Royal RE, Overman MJ, Rashid A, Abraham SC. High-level microsatellite instability in appendiceal carcinomas. Am J Surg Pathol. 2013;37(8):1192–200. doi:10.1097/PAS.0b013e318282649b.
22. Zauber P, Berman E, Marotta S, Sabbath-Solitare M, Bishop T. Ki-ras gene mutations are invariably present in low-grade mucinous tumors of the vermiform appendix. Scand J Gastroenterol. 2011;46(7–8):869–74. doi:10.3109/00365521.2011.565070.
23. Liu X, Mody K, de Abreu FB, Pipas JM, Peterson JD, Gallagher TL, Suriawinata AA, Ripple GH, Hourdequin KC, Smith KD, Barth RJ Jr, Colacchio TA, Tsapakos MJ, Zaki BI, Gardner TB, Gordon SR, Amos CI, Wells WA, Tsongalis GJ. Molecular profiling of appendiceal epithelial tumors using massively parallel sequencing to identify somatic mutations. Clinical chemistry. 2014;60(7):1004–11. doi:10.1373/clinchem.2014.225565.
24. Alakus H, Babicky ML, Ghosh P, Yost S, Jepsen K, Dai Y, Arias A, Samuels ML, Mose ES, Schwab RB, Peterson MR, Lowy AM, Frazer KA, Harismendy O. Genome-wide mutational landscape of mucinous carcinomatosis peritonei of appendiceal origin. Genome medicine. 2014;6(5):43. doi:10.1186/gm559.
25. Tsai JH, Yang CY, Yuan RH, Jeng YM. Correlation of molecular and morphological features of appendiceal epithelial neoplasms. Histopathology. 2019;75(4):468–77. doi:10.1111/his.13924.
26. LaFramboise WA, Pai RK, Petrosko P, Belsky MA, Dhir A, Howard PG, Becich MJ, Holtzman MP, Ahrendt SA, Pingpank JF, Zeh HJ, Dhir R, Bartlett DL, Choudry HA. Discrimination of low- and high-grade appendiceal mucinous neoplasms by targeted sequencing of cancer-related variants. Modern

pathology: an official journal of the United States Canadian Academy of Pathology Inc. 2019;32(8):1197–209. doi:10.1038/s41379-019-0256-2.

27. Zhu X, Salhab M, Tomaszewicz K, Meng X, Mathew C, Bathini V, Switzer B, Walter O, Cosar EF, Wang X, Lambert LA, Hutchinson LM. Heterogeneous mutational profile and prognosis conferred by TP53 mutations in appendiceal mucinous neoplasms. Human pathology. 2019;85:260–9. doi:10.1016/j.humpath.2018.11.011.
28. Pai RK, Hartman DJ, Gonzalo DH, Lai KK, Downs-Kelly E, Goldblum JR, Liu X, Patil DT, Bennett AE, Plesec TP, Kuan SF, Nikiforova MN, Shadrach B, Pai RK. Serrated lesions of the appendix frequently harbor KRAS mutations and not BRAF mutations indicating a distinctly different serrated neoplastic pathway in the appendix. Human pathology. 2014;45(2):227–35. doi:10.1016/j.humpath.2013.10.021.

Tables

Table 1 Clinicopathologic features of patients with appendiceal lesions

Clinicopathologic features	LAMN	Serrated lesions
Total no. of cases	13	12
Gender(F/M)	11/2	7/5
Average age	63.5	66.7
Lesion size (mm)(range)	2x1x1cm-4.2x1.5x1.5cm	0.3cm-4.7x0.6x0.6cm
Gross cystic dilatation	8	0
Gross luminal mucin	13	12
Gross rupture	4	0
Polyp growth pattern	1	2
Discrete polyp	1	2
Associated lesions		
Colon carcinoma	3	4
IBD (UC or CD)	0	0
Overy carcinoma	0	3
PMP	9	0
Appendicitis	1	1

IBD: inflammatory bowl disease; UC: ulcerative colitis; CD: Crohn’s disease; PMP:pseudomyxoma peritonei

Table 2 Immunohistochemical features of study patients with appendiceal lesions

Immunohistochemical features	LAMN	Serrated lesions	P Value
Total no. of cases	13	12	
CK20	Diffuse positive, 3 with crypt scatter positive	Diffuse positive, 10 with crypt scatter positive	0.005*
MUC6	5 focal positive	6 focal or scatter positive	0.695
MUC5AC	13 diffuse positive	11 diffuse positive	0.458
MUC1	12 focal surface epithelium positive	4 focal surface epithelium positive	0.008*
P53	6 positive	0 positive	0.015*
Ki-67	4 case positive both in the crypt and in surface epithelium, 9 with crypt positive	1 case irregular positive, 11 cases with crypt positive	0.322

P Vaue was calculated using $\chi 2$ test.

* statistically significant different

Table 3 Immunohistochemical and molecular Micro-satellite state

	LAMN	Serrated lesions	P Value
Total case	13	12	
dMMR	3	7	0.047*
PMS2	All preserved	8 preserved, 4 deficient	
MHL1	All preserved	3 deficient	
MSH6	3 deficient	8 deficient	
MSH2	All preserved	All preserved	
MSI-DNA			1.000*
MSS	12	11	
MSI-H	0	0	
MSI-L	1	1	

P Vaue was calculated using $\chi 2$ test.

* statistically significant different

Table 4 Molecular features of study patients with appendiceal lesions

molecular features	LAMN	Serrated lesions	P Value
Total case	13	12	
K-RAS(Cases /Mutation type)	12	5	0.011*
G12V/35G>T	2(1case co-exist GNAS mutation)	0	
G12D/35G>A	8(3cases co-exist GNAS mutation)	2(1 case co-exist GNAS mutation)	
G13D/38G>A	2(1 case co-exist GNAS mutation)	2(1 case co-exist GNAS mutation)	
G12C/34G>T	0	1	
No mutaion	1	7	
B-RAF	0	3(with no other mutation)	0.096
G-NAS(Cases /Mutation type)	5	2	0.378
602G□A	2	2	
601C□T	3	0	
No mutation	8	10	

P Vaue was calculated using χ2 test.

* statistically significant different

Figures

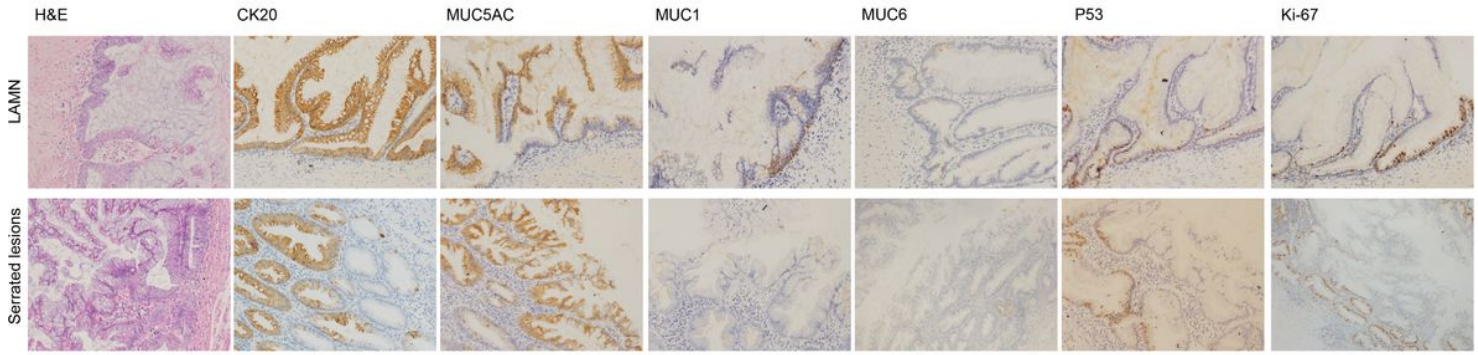

Figure 1

LAMN and serrated lesions of appendix. Both two lesions showed diffuse CK20 expression. Scattered CK20 positive in deep crypt could be seen in serrated lesion. MUC1 and MUC6 could be detected locally in LAMN and serrated lesions. MUC5AC were diffuse positive in LAMN and serrated lesion, both in the crypt and in surface epithelium. P53 and Ki67 were positive only in base of crypt.

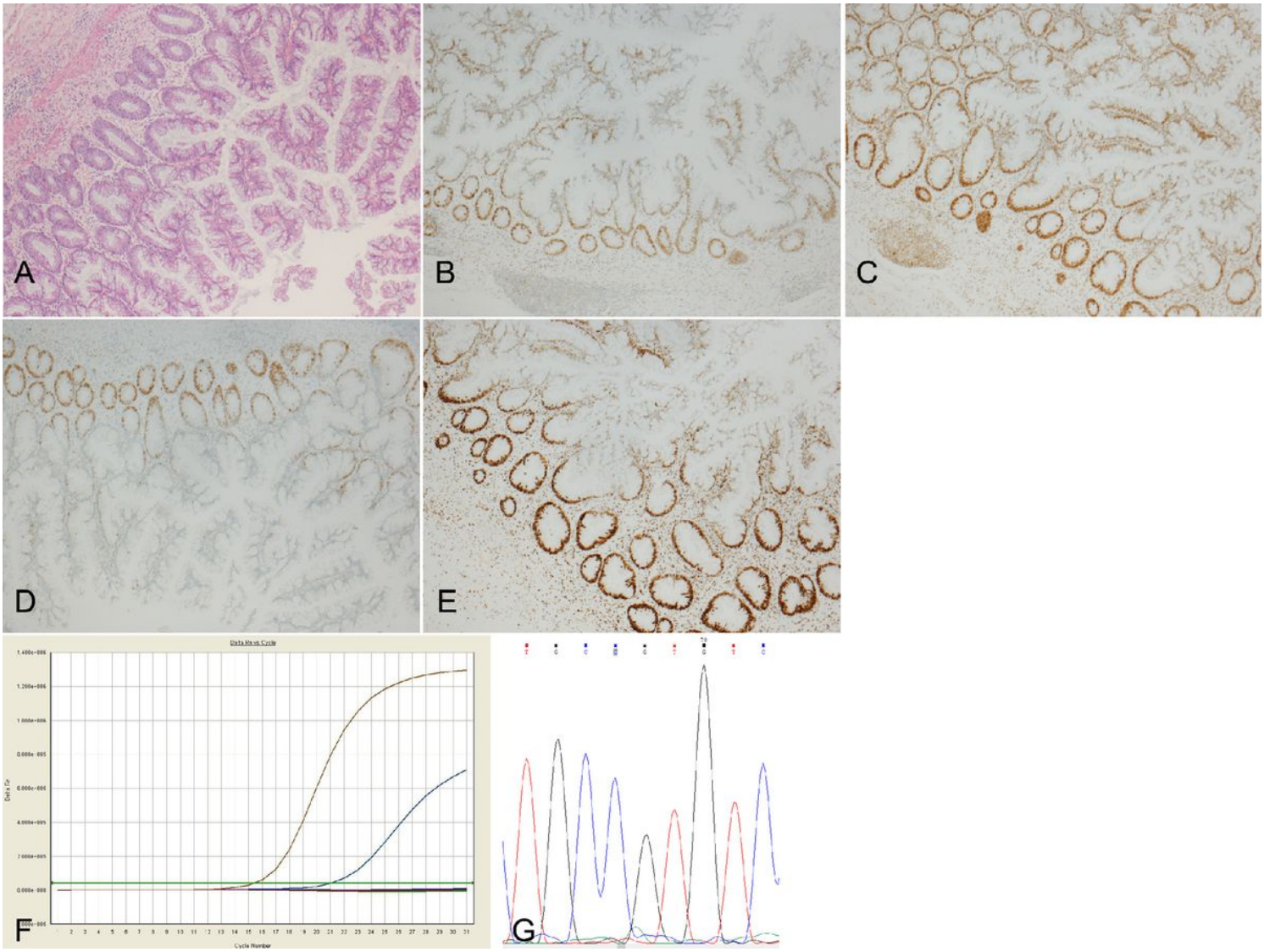

Figure 2

A H&E stain of a case of serrated lesion (40x). B MLH1 staining positive in tumour. C Positive MSH2 staining. D MSH6 negative in tumour cells. E Positive PMS2 staining. F Real-time PCR (ARMS sequencing) showed G13D mutation in K-ras. G Sanger sequencing showed 602 G□A mutation in GNAS.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable5.7.doc