Published in final edited form as:

Am J Surg Pathol. 2016 January ; 40(1): 113–119. doi:10.1097/PAS.0000000000000531.

Loss of Hes1 Differentiates Sessile Serrated Adenoma/polyp from Hyperplastic Polyp

Min Cui, MD, PhD^{1,2}, Amad Awadallah, BSc¹, Wendy Liu, MD, PhD¹, Lan Zhou, MD, PhD^{1,2}, and Wei Xin, MD, PhD^{1,2,#}

¹Department of Pathology, University Hospitals Case Medical Center, Cleveland, OH 44106

²Department of Pathology, Case Western Reserve University, Cleveland, OH, 44106

Abstract

Sessile serrated adenoma/polyp (SSA/p) is a precancerous lesion, and its differential diagnosis from hyperplastic polyp (HP) could be challenging in certain circumstances based on morphology alone. Hes1 is a downstream target of Notch signaling pathway and plays an important role in intestinal development by regulating differentiation of enterocytes. In this study, we evaluated the expression patterns of Hes1 in SSA/p and hyperplastic polyp (HP), and determine whether Hes1 immunostaining can help differentiate between these two entities. Serrated polyps with cytological dysplasia (sessile serrated adenoma with cytological dysplasia, tubular adenoma, and traditional serrated adenoma) were also studied. Hes1 is ubiquitously expressed in the nuclei of normal colon epithelial cells. The complete loss or a very weak expression of Hes1 is observed in the majority of the SSA/p in the study (58/63, 92%) compared to the normal expression of Hes1 in HP (35/35, 100%). In SSA/p with cytological dysplasia, dysplastic area demonstrated cytoplasmic and/or nuclear staining for Hes1. Tubular adenoma and traditional serrated adenoma showed variability of Hes1 staining within the polyp with a mixed positive and negative staining pattern. Our study suggests that loss of Hes1 could be used as a sensitive and specific marker to differentiate SSA/p from HP, which helps the diagnosis in morphologically challenging cases.

Keywords

sessile serrated adenoma/polyp; hyperplastic polyp; Hes1; Notch; immunohistochemistry

Introduction

Sessile serrated adenoma/polyp (SSA/p) is a recently recognized precursor lesion for colorectal carcinoma, mostly located in the right side colon (cecum, ascending colon, and transverse colon).¹ The detailed diagnostic criteria have also been evolved since the establishment of this diagnostic entity. Two to three contiguous SSA/p-type crypts are sufficient for the diagnosis of SSA/p according to the fourth edition of the WHO Classification of Tumors of the Digestive System,² while the 2012 consensus meeting recommend that serrated polyps with as few as one SSA-type crypt should be diagnosed as

#Correspondence: Wei Xin, MD, PhD, Department of Pathology, University Hospitals Case Medical Center, Case Western Reserve University, 10900 Euclid Ave, Cleveland, OH 44106, (wei.xin@uhhospitals.org).

SSA/p.³ Sometimes being limited by the size, orientation and other biopsy artifacts, the differential diagnosis of SSA/p from hyperplastic polyp (HP) could be morphologically challenging based on the histology only. Especially, in the left colon, prolapse colonic mucosa can mimic SSA/p, which has been recognized and described by Huang et al.⁴ Interobserver agreement for the diagnosis of SSA/p has been moderate based on hematoxylin and eosin (H&E) staining.⁵ The distinction between SSA/p and HP is clinically important because they have different recommended surveillance interval based on their apparently different cancer risk.⁶ A sensitive and specific marker for SSA/p would be helpful to diagnose morphologically challenging cases. Biomarkers for SSA/p have been explored and studied. MUC6 has been reported to be expressed in SSA/p but not in HP.⁷ However, MUC6 was found to lack specificity for SSA/p in another study.⁸ The immunohistochemistry scoring of annexin A10, encoded by ANXA10, was reported to carry a sensitivity of 73% and specificity of 95% in the diagnosis of SSA/p.⁹ Ideal biomarkers with both high sensitivity and specificity are still lacking.

Notch signaling plays essential functions in intestinal development and homeostasis. Hes1 is a well-known downstream target of Notch signaling pathway and plays an important role in intestinal development by regulating differentiation of enterocytes. Hes1 is expressed in the nuclei of epithelial cells of the intestine in both human and mice, and its expression has been shown to be increased in adenoma and colon adenocarcinoma.¹⁰ The expression of Hes1 is also shown associated with the differentiation of colon adenocarcinoma, with a stronger expression of Hes1 represented in poorly differentiated adenocarcinoma.¹¹ However, the expression of Hes1 in colon adenocarcinoma shown in the literature is not consistent. In a paper published by Fre et al, Hes1 is strongly expressed in adenomas but not expressed in 14 cases of human colon adenocarcinomas, indicating that loss of Hes1 could be associated with carcinogenic transformation of adenoma.¹² A study performed by Ueo et al showed that in intestinal specific Hes1 knockout mice, greatly expanded crypts were observed in the colon by 2 months of age.¹³ Our own studies of an animal model with genetic defects in Notch signaling indicate that suppressed Hes1 expression in this model is associated with the development of SSA/p-like polyps and then adenocarcinoma in mouse colon (unpublished data). Based on our own findings and reports from others, we speculated that loss of Hes1 can be a promising marker for SSA/p. Here in this study, we have studied Hes1 expression in SSA/p and HP as well as other serrated polyps, including SSA/p with cytological dysplasia, traditional serrated adenoma (TSA) and tubular adenoma (TA).

Material and Methods

Sample Collection

H&E stained sections from a single tertiary care medical center were reviewed. All the colon biopsies were performed from 2013 to 2014. All specimens analyzed were formalin-fixed and paraffin-embedded tissue sections. All the cases were reviewed by the junior pathologist, and then independently reviewed by two GI subspecialty practicing pathologists. Only the cases with consensus diagnosis among 3 pathologists were included in the study. A total of 63 SSA/p (41-81 years old, mean age 62.3 years old, male/female ratio 1.0) and 35 HP (39-85 years old, mean age 62.6 years old, male/female ratio 1.2) were

selected. Among the 63 SSA/p cases, 45 were biopsies from the right colon (cecum and ascending colon), and 18 were from the left colon (descending colon, sigmoid colon and rectum). For the 35 HP, 27 were from the left colon and 8 were from the right colon. The HPs were subdivided into the microvesicular subtype (n=28) and the goblet cell rich subtype (n=7) based on mucin distribution.¹ No mucin-poor HP was included in the study. Fifteen TAs, 10 TSA and 3 SSA/p with dysplasia were included in the study, respectively. The study was approved by the Institutional Research Board (IRB) of the University Hospitals Case Medical Center. Written consent was waived since the study was carried on discarded tissues and involves chart-review only.

Immunohistochemical (IHC) Staining of Hes1

Expression of Hes1 was evaluated by immunohistochemistry (IHC) on all cases included in this study. The IHC was performed by the Immunohistochemistry Diagnostic Laboratory of University Hospitals Case Medical Center. Tissue slides were processed using a BenchMark Ultra automated immunostainer (Ventana, Tucson, AZ). Slides were deparaffinized, antigen retrieved with standard Cell Conditioning 1 (Ventana Medical Systems, AZ), a tris-based buffer pH 8.3 solution for 64 minutes at 95 C, then incubated at 37 C with the primary antibody HES1 rabbit monoclonal (1:200 dilution, Catalog #2922-1, clone EPR4226 from Epitomics CA., USA., part of Abcam, Cambridge, MA) for 24 minutes. Detection was performed with Ultraview-DAB from Ventana (Tucson, AZ) and subsequently counterstained.

Statistical Analysis

Comparison of the Hes1 expression rates among HP and SSA/p was done using the Fisher's exact test (two-tailed).

Results

Hes1 Expression in Normal Colon and Hyperplastic Polyp

As shown in Figure 1A-B, in normal colonic mucosa, Hes1 IHC demonstrated consistent nuclei staining in surface epithelial cells, crypt cells and interstitial inflammatory cells in the lamina propria. We observed strong and consistent nuclear staining in the interstitial inflammatory cells in the lamina propria in every case in this study. Hes1 staining intensity comparable to lamina propria cells is interpreted as positive expression. In HP cases, nuclei staining of Hes1 was maintained in the crypts in both microvesicular (28/28, 100%, Figure 1C-D) and goblet cell (7/7, 100%, Figure 1E-F) hyperplastic polyps. The staining intensity is comparable to normal colon mucosa. The nuclear expression of Hes1 was also maintained in the distorted crypts of hyperplastic polyp with prolapse mucosa (Figure 1 G-H).

Hes1 Expression in SSA/p and SSA with Dysplasia

As shown in Figure 2 A-B, loss of or very weak Hes1 expression in the crypts was identified in the majority of SSA/p cases studied (53/58). There are five SSAs with positive nuclei staining in the crypts, all located in the left colon (three in sigmoid colon and two in rectums, images not shown). A summary of the Hes1 immunohistochemistry results of HP and SSA/p according to the sites of the polyps is provided in the Table 1. There is significant

difference ($P<0.001$) in positive Hes1 staining for HP and SSA/p when comparison is performed based either on the diagnosis (SSA verse HP), or based on the site of polyps (left sided SSA verse HP, right sided SSA verse HP).

Hes1 has a unique staining pattern in SSA with cytological dysplasia. Instead of showing a complete loss or very weak staining in the nuclei, it displays a diffuse positive staining in the cytoplasm in the dysplastic epithelium (Figure 2 C-D). This staining pattern was observed in all cases of SSA with dysplasia.

Hes1 Expression in Tubular Adenoma (TA) and Traditional Serrated Adenoma (TSA)

In our study, Hes1 expression in TA was variable within the polyp, with mostly robust positive nuclear staining but loss of Hes1 expression in some patchy areas (Figure 3 A-B). In TSA, Hes1 expression also showed a mixed pattern within the polyp, with focal strong nuclear staining and focal loss of expression (Figure 3 C-D). The staining of Hes1 in the lamina propria cells in these polyps was uniform and robust.

Discussion

The diagnosis of SSA/p could be challenging due to the heavy reliance on the morphology of basal crypts and the interobserver variability. A biomarker that is easy to perform and interpret in daily practice would help practicing pathologists in morphological challenging cases. In this study we explored the utility of the immunostaining of a Notch downstream target transcriptional factor, Hes1, in differentiating SSA/p from HP. As shown in table 1, loss of Hes1 expression has a sensitivity of 92% and specificity of 100% in differentiating SSA/p from HP. HP with mucosal prolapse is recognized to be a mimicker of SSA. Our study showed that nuclear staining of Hes1 is maintained in the distorted crypts of this condition. Therefore, the immunostaining of Hes1 could be particularly helpful to diagnose polyps from sites prone to mucosal prolapse condition. We confirmed the reported finding that Hes1 is normally expressed in the nuclei of epithelium of colorectal mucosa. We also observed a strong nuclear expression of Hes1 in interstitial inflammatory cells in lamina propria, which can serve as a universal internal positive control and reference level for each specimen. The nuclear staining pattern is also a favorable factor for interpretation compared to the cytoplasmic staining.

A small number of polyps in this study located in the left colon have morphology consistent with SSA/p but show positive nuclear staining of Hes1. This suggests two possibilities: one possibility is that the left-sided SSA/p could maintain Hes1 expression, while right-sided SSA/p normally shows uniform loss of Hes1. It has been reported that distinct genetic and epigenetic features exist in the proximal SSA/p and the distal SSA/p.¹⁴ Another possibility is that these left-sided polyps are in fact hyperplastic polyps with morphology indiscernible from SSA/p due to various factors (such as orientation, processing artifact etc.). Further study with more definitive standard is needed to clarify this issue, as the current biomarkers such as BRAF mutation is not specific enough to distinguish SSA/p from HP.¹⁵

Hes1 is shown to be associated with secretory lineage differentiation of intestinal epithelial cells.¹³ It is unclear yet at which level (transcriptional or translational) the down regulation

of Hes1 takes place in the SSA/p. If it occurs at the transcriptional level, the consistent expression of Hes1 in interstitial inflammatory cells in lamina propria would make it difficult to identify Hes1 as a differentially expressed genes by cDNA microarray approach or methylation study using an entire polyp. An in situ RNA hybridization would be helpful to clarify whether down regulation of Hes1 in SSA/p is regulated transcriptionally; however this is beyond the scope of the current study which focuses on the IHC staining of Hes1.

TA and TSA are common malignancy precursors encountered in colorectal biopsies. Although not the focus of this study, we evaluated limited number of cases to gain basic knowledge of Hes1 staining pattern in these polyps. TA adenoma has upregulated Hes1 expression according to previous reports. We have observed a mixed staining pattern in the cases we studied, with mostly positive nuclear staining and some focal negative staining shown for TA, and mixed staining pattern for TSA. Given that there is a robust and uniform staining of Hes1 in lamina propria inflammatory cells, the mixed staining pattern is unlikely to be an artifact. This is consistent with the notion that TA is an epigenetically diverse population.¹⁶ TSA has also been shown to be a genetically heterogeneous group, with both KRAS and BRAF mutations commonly identified.¹⁷ Interestingly, in the dysplastic epithelium of SSA/p, we observed a diffuse positive staining of Hes1 in the cytoplasm suggestive of altered location of Hes1 and hence possible dysregulation of Hes1 signaling. We speculate that this finding may be related to genetic and/or epigenetic changes with SSA progression to SSA with dysplasia, and more in-depth study would be needed to investigate the underlying mechanism. The distinction between these three types of adenomas with cytological dysplasia could potentially be helped by Hes1 IHC based on the unique staining pattern of dysplastic portion of SSA, although a more extensive study with larger number of cases included in each category would be needed before we could reach the conclusion.

Conclusion

In this study, we found that Hes1 expression is either completely lost or shows weak staining in SSA/p compared to HP or normal colonic mucosa. Loss of Hes1 has the potential as a useful marker for diagnosing SSA/p in morphologically challenging cases. We also observed that Hes1 expression is switched to positive cytoplasmic staining in dysplastic epithelium in SSA/p with cytological dysplasia. Tubular adenoma and traditional serrated adenoma showed variable Hes1 staining within the polyp with a mixed positive and negative staining pattern in nuclei.

Acknowledgment

Part of the manuscript was presented as an abstract form in 104th (2015) USCAP annual meeting at Boston, MA. The research was partially supported by the small research grant from the Department of Pathology at University Hospital Case Medical Center; and the faculty startup fund (Dr. Wei Xin) from Case Western Reserve University.

References

1. Torlakovic E, Skovlund E, Snover DC, Torlakovic G, Nesland JM. Morphologic reappraisal of serrated colorectal polyps. *Am J Surg Pathol*. 2003; 27:65–81. [PubMed: 12502929]

2. Bosman, FT.; World Health Organization. International Agency for Research on Cancer. WHO classification of tumours of the digestive system. International Agency for Research on Cancer; Lyon: 2010. p. 417
3. Rex DK, Ahnen DJ, Baron JA, Batts KP, Burke CA, Burt RW, Goldblum JR, Guillem JG, Kahi CJ, Kalady MF, O'Brien MJ, Odze RD, Ogino S, Parry S, Snover DC, Torlakovic EE, Wise PE, Young J, Church J. Serrated lesions of the colorectum: review and recommendations from an expert panel. *Am J Gastroenterol*. 2012; 107:1315–1329. quiz 1314, 1330. [PubMed: 22710576]
4. Huang CC, Frankel WL, Doukides T, Zhou XP, Zhao W, Yearsley MM. Prolapse-related changes are a confounding factor in misdiagnosis of sessile serrated adenomas in the rectum. *Hum Pathol*. 2013; 44:480–486. [PubMed: 23069257]
5. Glatz K, Pritt B, Glatz D, Hartmann A, O'Brien MJ, Blaszyk H. A multinational, internet-based assessment of observer variability in the diagnosis of serrated colorectal polyps. *Am J Clin Pathol*. 2007; 127:938–945. [PubMed: 17509991]
6. Lieberman DA, Rex DK, Winawer SJ, Giardiello FM, Johnson DA, Levin TR. Guidelines for colonoscopy surveillance after screening and polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer. *Gastroenterology*. 2012; 143:844–857. [PubMed: 22763141]
7. 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. *Mod Pathol*. 2008; 21:660–669. [PubMed: 18360351]
8. Gibson JA, Hahn HP, Shahsfaei A, Odze RD. MUC expression in hyperplastic and serrated colonic polyps: lack of specificity of MUC6. *Am J Surg Pathol*. 2011; 35:742–749. [PubMed: 21490447]
9. Gonzalo DH, Lai KK, Shadrach B, Goldblum JR, Bennett AE, Downs-Kelly E, Liu X, Henricks W, Patil DT, Carver P, Na J, Gopalan B, Rybicki L, Pai RK. Gene expression profiling of serrated polyps identifies annexin A10 as a marker of a sessile serrated adenoma/polyp. *J Pathol*. 2013; 230:420–429. [PubMed: 23595865]
10. Peignon G, Durand A, Cacheux W, Ayrault O, Terris B, Laurent-Puig P, Shroyer NF, Van Seuningen I, Honjo T, Perret C, Romagnolo B. Complex interplay between beta-catenin signalling and Notch effectors in intestinal tumorigenesis. *Gut*. 2011; 60:166–176. [PubMed: 21205878]
11. Gao F, Zhang Y, Wang S, Liu Y, Zheng L, Yang J, Huang W, Ye Y, Luo W, Xiao D. Hes1 is involved in the self-renewal and tumorigenicity of stem-like cancer cells in colon cancer. *Sci Rep*. 2014; 4:3963. [PubMed: 24492635]
12. Fre S, Pallavi SK, Huyghe M, Lae M, Janssen KP, Robine S, Artavanis-Tsakonas S, Louvard D. Notch and Wnt signals cooperatively control cell proliferation and tumorigenesis in the intestine. *Proc Natl Acad Sci U S A*. 2009; 106:6309–6314. [PubMed: 19251639]
13. Ueo T, Imayoshi I, Kobayashi T, Ohtsuka T, Seno H, Nakase H, Chiba T, Kageyama R. The role of Hes genes in intestinal development, homeostasis and tumor formation. *Development*. 2012; 139:1071–1082. [PubMed: 22318232]
14. Maeda T, Suzuki K, Togashi K, Nokubi M, Saito M, Tsujinaka S, Kamiyama H, Konishi F. Sessile serrated adenoma shares similar genetic and epigenetic features with microsatellite unstable colon cancer in a location-dependent manner. *Exp Ther Med*. 2011; 2:695–700. [PubMed: 22977561]
15. Fujita K, Yamamoto H, Matsumoto T, Hirahashi M, Gushima M, Kishimoto J, Nishiyama K, Taguchi T, Yao T, Oda Y. Sessile serrated adenoma with early neoplastic progression: a clinicopathologic and molecular study. *Am J Surg Pathol*. 2011; 35:295–304. [PubMed: 21263251]
16. Humphries A, Cereser B, Gay LJ, Miller DS, Das B, Gutteridge A, Elia G, Nye E, Jeffery R, Poulsom R, Novelli MR, Rodriguez-Justo M, McDonald SA, Wright NA, Graham TA. Lineage tracing reveals multipotent stem cells maintain human adenomas and the pattern of clonal expansion in tumor evolution. *Proc Natl Acad Sci U S A*. 2013; 110:E2490–2499. [PubMed: 23766371]
17. Wiland, HOt; Shadrach, B.; Allende, D.; Carver, P.; Goldblum, JR.; Liu, X.; Patil, DT.; Rybicki, LA.; Pai, RK. Morphologic and molecular characterization of traditional serrated adenomas of the distal colon and rectum. *Am J Surg Pathol*. 2014; 38:1290–1297. [PubMed: 25127095]

Figure 1.

Hes1 expression in normal colon mucosa and HP. Nuclear staining of Hes1 is present in both surface epithelial cells and crypt cells in normal colon mucosa (A-B), hyperplastic polyp microvesicular subtype (C-D, low power 2x objective for view of the entire polyp; E-F, 10x objective), goblet cell rich subtype (G-H). A, C, E, and G: Hematoxylin and eosin staining; B, D, F, and H: Hes1 immunohistochemistry.

Figure 2.

Hes1 expression in hyperplastic polyp with prolapse mucosa. Nuclear staining of Hes1 is preserved in distorted crypt cells (C, 10x objective; D, 20 x objective). A, B: Hematoxylin and eosin staining at 4x and 10x objective; C, D: Hes1 immunohistochemistry.

Figure 3.

Hes1 expression in SSA/p and SSA/p with dysplasia. Hes1 is lost or very weak in crypts from sessile serrated adenoma/polyp (A-B from one SSA, C-F from another SSA, with higher magnification in E, F to show some cells in SSA crypts have very weak staining compared to an adjacent crypt with positive Hes1 staining). Positive Hes1 staining in cytoplasm is present in dysplastic region of sessile serrated adenoma with dysplasia. Transitional area between dysplastic region and sessile serrated adenoma is shown (G-H). A, C, E, and G: Hematoxylin and eosin staining; B, D, F, and H: Hes1 immunohistochemistry 10x objective. A-D, G, H, 10x objective; E, F, 20x objective.

Figure 4. Hes1 expression in TSA and TA. Hes1 showed mixed positive and negative staining in tubular adenoma (A-B) and traditional serrated adenoma (C-D). A, C: Hematoxylin and eosin staining; B, D: Hes1 immunohistochemistry. 10 x objectives.

Table 1

Hes1 staining in hyperplastic polyp and sessile serrated adenoma/polyp based on anatomic location.

Polyp type	Right colon	Left colon	Total
	Positive, n (%)	Positive, n (%)	Positive, n (%)
HP	8/8 (100%)	27/27 (100%)	35/35 (100%)
SSA/p	0/45 (0%) [*]	5/18 (28%) [*]	5/63 (8%) [*]
Total	53	45	98

^{*} P<0.0001 between hyperplastic polyp and sessile serrated adenoma when compared the total group or separate group based on the location in right or left colon.