Original Article

Evaluation of Hairy and Enhancer of Split(HES-1) Expression in Different Types of Colorectal Polyps

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ABSTRACT

Background:
Colorectal cancer (CRC) is considered to be the fourth leading cause of cancer death worldwide.
Objectives: This study aimed to investigate the Hairy and Enhancer of Split-1 (HES-1) expression in different types of colorectal polyps.

Materials and Methods:
In this analytical study, we included 100 patients with colorectal polyps who were referred to Tehran, Iran. HES-1 immunostaining was performed to evaluate nuclear and/or cytoplasmic staining for HES-1 in the specimens. Then, the data were analyzed in terms of age, sex, side location, and polyp type.

Results:
Out of 100 subjects, 62 were men and 38 were women. The highest prevalence of polyps was observed in the rectum (34%), and the most common type of polyp was tubular adenoma (40%). HES-1 index was only positive in 22 cases. There was a significant relationship between HES-1 positivity and both polyp location (P<0.001), polyp type (P<0.001), and grade of dysplasia (P=0.019). However, there was no significant relationship between HES-1 positivity and adenomatous polyp type (P=0.52). The distribution of HES-1 positivity on the left side was significantly higher, and the relationship between HES-1 positivity and side location was significant (P=0.002).

Conclusion:
This study suggests that HES-1 can be useful in differentiating SSA/P and HP with high sensitivity and specificity.

Keywords: Polyp; Colon; Pathology; Immunohistochemistry markers; HES-1

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INTRODUCTION

Cancer is highly prevalent and causes numerous complications in patients. Complications of cancer include reduced life expectancy, increased stress and anxiety, decreased mental health, increased costs for patients, and the pressure of caregiving and disability (1-5). CRC is considered to be the fourth leading cause of cancer (6,7). CRC has high incidence rates among both sexes. Studies have suggested a significant association between various types of colorectal polyps and CRC (8,9). Recent studies in the United States have shown that the implementation of colonoscopy allows for the early detection and removal of precancerous polyps, which leads to a large decline in the incidence rate of colorectal malignancies. However, there is also abundant epidemiological evidence to suggest that the incidence rate of CRC is substantially increasing in Eastern Europe and Asia (8,10,11).

Gastrointestinal (GI) tract cancer is reported as the third most common cancer in the Iranian population, and it appears to occur in younger generations. Although the studies on upper GI cancers in Caspian Littoral in Iran are appropriately investigated, the CRC studies were neglected due to lower incidence in the 1970s. Nevertheless, the shift in the direction of prevailing rates may be motivating for further evaluations (12,13).

The overall risk of the development of adenomas and CRC is influenced by both genetic and epigenetic alterations (13-16). Adenomatous polyps have been known to ultimately go on to develop colorectal adenocarcinoma. It is estimated that the progression of these precursor lesions to malignant neoplasms takes 10 years on average (14,17). On the other hand, relying heavily upon morphology to differentiate Sessile serrated adenoma/polyp (SSA/P) from hyperplastic polyp (HP) could be challenging due to the reasons including size and orientation of the specimens in addition to other biopsy artifacts (18).

Immunohistochemistry (IHC) markers are generally one of the important factors in prognosis and survival (19, 20). Translation factor Hairy and Enhancer of Split-1 (HES-1) is a protein encoded by the HES-1 gene (21), and it is one of seven members of the HES gene family that encode transcriptional repressor proteins(22, 23). HES-1 marker is the most characterized target of Notch molecular signaling pathways, which functions in intestinal development by regulating the differentiation of enterocytes (18). In fact, activation of the Notch pathway releases the Intracellular Domain and transfers it into the nucleus(24).

Based on a previous study done by Sail and colleagues in 2013, a small molecule of compound 3 can induce HES-1 expression. The authors concluded it can be studied further as a potential HES-1 modulator and anticancer chemotherapeutics (25). Gao and others also demonstrated that HES-1 could be recognized as a self-renewal marker in colon cancer. It showed some properties of stem-like cells, which leads to tumor formation in mice (26).

In 2017, Wu and co-workers showed the correlation of hypomethylation of HES-1 in intestinal stem and progenitor cells. They finally cultivate the idea that hypomethylation of HES-1 plays a critical role in the prognosis of CRC patients (27). Yuan and colleagues conducted an investigation on 320 CRC samples and determined that HES-1 induces mesenchymal-epithelial transition, enhances the invasiveness of tumor cells, and promotes cancer metastasis (28). Weng and others proposed that HES-1 is a predictor of poor prognosis factors of CRC, and its overexpression can increase the invasion ability of CRC (29).

In another study conducted by Ahadi and others, IHC for HES-1 was performed on 2775 consecutive cases with colorectal adenocarcinomas. 36.1% of cases demonstrated cytoplasmic staining, while 17% of them were positive for nuclear staining. They concluded the latter group was significantly associated with factors such as female sex, older age, larger size of the tumor, and right-side location (30). Cui and colleagues evaluated the expression of HES-1 in both HPs and SSA/Ps and found that the expression of HES-1 was present in all HPs compared with SSA/Ps. Therefore, they suggested HES-1 as a diagnostic tool that helps differentiate between these two types of polyps (18).

Several studies have demonstrated over-expression of HES-1 in human CRCs (18,20,21,24,25,26). Despite the remarkable progress made so far, there is still little information available on the nuclear expression of this marker in different types of polyps, particularly adenomatous polyps, as a well-defined risk factor for CRC (13) (31) .

Objectives This study aimed to investigate the HES-1 expression in different types of colorectal polyps and determine their association with the clinicopathological status in patients with CRC.

MATERIALS AND METHODS

In this analytical study, we included the patients who underwent colonoscopy in the teaching hospitals affiliated with Shahid Beheshti University of Medical Sciences, Tehran, Iran. All colon polyp biopsy samples were gathered from April 2018 to April 2019. This research was carried out after the approval of the Ethics Committee of Shahid Beheshti University of Medical Sciences and the ethical standards were ensured throughout the procedures. A total of 100 patients were selected, and the required information was obtained, such as age, sex, side location, and polyp type. The exclusion criteria are as follows: invalidated

Table 1. Frequency of sex and HES-1 expression in different age groups of the patients

Variable		Age groups (years, N, %)					Total
		Less than 40	40-49	50-59	60-69	More than 70	
Sex	Male	6 (9.7)	4 (6.5)	15 (24.2)	20 (32.3)	17 (27.4)	62 (100)
	Female	4 (10.5)	10 (26.3)	12 (31.6)	8 (21.1)	4 (10.5)	38 (100)
HES-1	Positive	2 (9.9)	5 (22.7)	7 (31.8)	5 (22.7)	3 (13.6)	22 (100)
	Negative	8 (10.2)	9 (11.5)	20 (26.6)	23 (29.4)	18 (23)	78 (100)

immunohistochemical assays, patients who have received chemo/radiotherapy beforehand, and their reluctance to take part in the study.

Formalin-fixed and paraffin-embedded tissue specimens were reviewed in the pathology department of Shohada Tajrish Hospital and Shahid Modarres Hospital, both located in Tehran, and then expression of HES-1 was evaluated by IHC on the included cases.

Paraffin blocks were initially cut into tissue sections and were kept at 37 °C for 24 hours. Then, the slides were washed with xylene and dehydrated ethanol, and the antigen was retrieved with retrieval PH 8 solution for 30 minutes, followed by incubating in a microwave oven. Subsequently, we let the slides cool to room temperature, washed with DDW, and placed them in hydrogen peroxide solution for 10 minutes. We added 100 landaus of primary antibody to the slides and incubated them for 30 minutes at 37 °C, then we washed them with phosphate-buffered staining (PBS) solution, added secondary antibody, and incubated them for another 15 minutes at room temperature. After another round of washing, Horseradish Peroxidase (HRP) solution was added, and the slides were incubated for 30 more minutes. We washed the slides one more time, and 3,3′-Diaminobenzidine (DAB) was utilized in the staining procedure. After one more wash with DDW, we counterstained them with hematoxylin for 30 seconds and successive washes with ethanol and xylene, with each wash lasting 3 minutes. Finally, we mounted the dried slides and visualized them under the microscope. Statistical analysis was performed using SPSS software version 24 for Windows (IBM Inc, NY).

RESULTS

This study was conducted on 100 patients (male: 62 and female:38) with CRC with a mean age of 59.13±13.64 years. The mean age of the men group was 61.40 ±13.30, and in the group of women, it was 55.42 ± 13.54. CRC was most seen in men aged 60-69 years (32.3%); among women, it was most common in the age group of 50-59 years (31.6%). There was a significant difference between the mean age of men and women in addition to the two sexes in terms of age group (P=0.034, P=0.023, respectively). Results demonstrated that HES-1 index was only positive in 22 cases. The mean age of patients with HES-1 expression was 55.86±13.04, and in other patients was 60.05±13.75 (P=0.19). HES-1 expression was not significantly different within two sex groups (P=0.28, Table 1).

As shown in Table 2, the highest prevalence of polyps was observed in the rectum (34%), and the most common type of polyp was tubular adenoma (40%). A significant relationship was found between HES-1 positivity and the location of their types (P=0.001, P=0.001, respectively); however, there was no significant relationship between HES-1 positivity and adenomatous polyp type (P=0.52). Furthermore, HES-1 was expressed in 32.8% of cases with a left-side polyp, compared with 4% of cases with a right-side polyp and no cases with polyps in the transverse colon. In addition, the analysis showed a significant relationship between HES-1 expression and the aspect of polyp (Table 2). Table 3 shows HES-1 nuclear expression in both HP and SSA/P. Of the total 36 cases of SSA/Ps and HPs, 15 polyps (41.6%) were SSA/Ps, and HPs comprised 21 cases (58.4%). All the cases were evaluated for HES-1 positivity afterward, and HES-1 expression was seemingly present in 76.2% of the HP group. While on the contrary, no cases in SSA/P group were positive for HES-1. Regarding HES-1 status in SSA/Ps and HPs, the P value could not be detected in the SSA/Ps due to the lack of HES-1 positive cases. Likewise, a significant relationship could not be found between HES-1 positivity and HPs either. However, 16 out of 36 polyps (44.4%) were HES-1 positive in both SSA/P and HP groups, and the positivity of this marker was significantly related to SSA/Ps and HPs overall (P<0.001) (Table 3, Figure 1). We also calculated the sensitivity of 76.2% and specificity of 100% in HES-1 expression as a diagnostic tool to differentiate between SSA/P and HP.

HES-1 expression in patients with high-grade and low-grade dysplasia yielded 28.6% and 4.1%, respectively. With regards to different types of adenomatous polyps, we found that HES-1 could not differentiate among them (P=0.52), yet the grade of dysplasia in these types of polyps and HES-1 positivity were significantly related (Table 4).

Table 2. HES-1 expression in terms of location, types, and aspects of the polyps

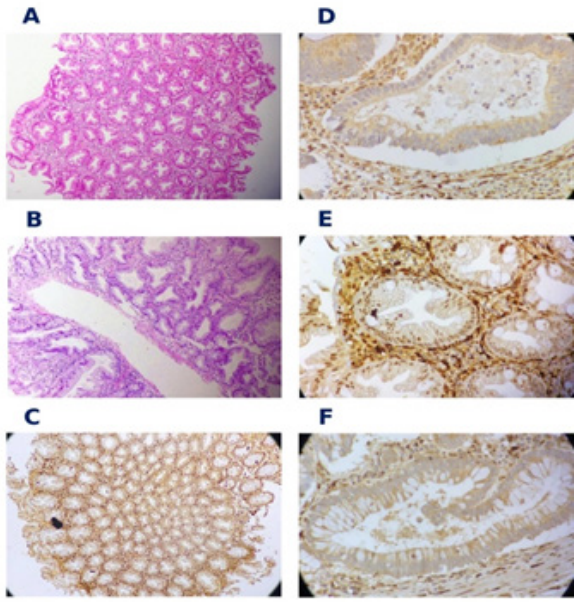
Variable		HES-1		Total
		Positive	Negative	
Location of polyps	Cecum	0	7	7
	Transverse colon	0	11	11
	Ascending colon	1	15	16
	Rectum	15	19	34
	Hepatic flexure	0	2	2
	Sigmoid	6	11	17
	Descending colon	0	13	13
Type of polyps	SSA(Sessile serrated adenoma)	0	15	15
	HP(Hyperplastic polyp)	16	5	21
	TA(Tubular adenoma)	5	35	40
	VA(Villous adenoma)	0	6	6
	TVA(Tubulovillous adenoma)	1	16	17
	TSA(Traditional serrated adenoma)	0	1	1
	SSA with dysplasia	0	1	1
Aspect of polyps	Right	1	24	25
	Left	21	43	64
	Transverse	0	11	11

Table 3. Distribution of HES-1 positive cases SSA/Ps and HPs in terms of polyp aspect

Variable			Aspect			Total
			Right	Left	Transverse	(%)
SSA/P	HES-1	Positive	0	0	0	0 (0)
		Negative	11	1	3	15 (100)
HP	HES-1	Positive	1	15	0	16 (76.2)
		Negative	1	3	1	5 (23.8)
Total	HES-1	Positive	1	15	0	16 (44.4)
		Negative	12	4	4	20 (55.6)

Table 4. HES-1 expression in high/low-grade dysplasia in different types of adenomatous polyps

Variable			Polyp type			Total
			TA (Tubular adenoma)	VA (Villous adenoma)	TVA (Tubulovillous adenoma)	
Dysplasia	High Grade	HES-1 positive	3	0	1	4
		HES-1 negative	3	5	2	10
	Low Grade	HES-1 positive	2	0	0	2
		HES-1 negative	32	1	14	47
Total		HES-1 positive	5	0	1	6
		HES-1 negative	35	6	16	57



Nuclear staining of HES-1 is preserved in crypt cells of HP (C, 10× objective; E, 40× objective) while it is lost in crypts from SSA/P (D, F, 40× objective). A, B: Hematoxylin and eosin staining; C, D, E, and F: HES-1 immunohistochemistry. A, B, C: 10×objective. D, E, F: 40× objective.

Figure 1. HES-1 Expression in HP and SSA/P

DISCUSSION

Colorectal polyps are known to have a risk of harboring cancer, and in some circumstances, diagnosis of these

polyps could be challenging on morphology alone. Therefore, studying molecular mechanisms and identifying the prognostic factors associated with the progression of CRC is of great value (32). HES-1 is considered to be one of these factors, and of note, most studies have examined its role in the incidence of CRC, and only a few studies have investigated its prognostic effect.

HES-1 positive cases were considered cell nucleus staining within the polyp, and the strong expression of HES-1 ubiquitously presented in the nuclei of inflammatory cells in the lamina propria was considered positive control.

Out of 100 patients included in the study, 62% were men, 38% were women, and HES-1 was found in 22 cases. Moreover, we found a significant relationship between the type of polyp and its location with HES-1 positivity. There was no association between HES-1 and adenomatous polyp type, but a significant relationship between the type of dysplasia and HES-1 positivity was shown earlier. The distribution of HES-1 positive cases on the left side was significantly higher, and the relationship between polyps and HES-1 positive cases was significant. Hyperplastic polyps were also related to HES-1 significantly. Regarding differentiating between SSA/P and hyperplastic polyp, sensitivity, specificity, PPV, and NPV were estimated at 76.19%, 100%, 100%, and 75%, respectively. As a result, examining HES-1 could have a good diagnostic value.

Our results showed only one case of SSA/P in the left side of colon which was not positive for HES-1. Although it is difficult to differentiate SSA/P and HP by morphologic features, left-sided polyps are mostly HPs (33). The study by Cui and colleagues showed that a small number of cases of colorectal polyps that are morphologically consistent with SSA/P are seen in the left colon, although, unlike our study, these cases were positive for HES-1. Therefore, unlike their study, the immunohistochemical study in our study showed the ability to detect SSA/P accurately.(18, 34).We studied 64 adenomatous polyps, including TA, VA, TVA, and TSA, in terms of HES-1 staining, and only six samples showed positive HES-1 nuclear staining, whereas the rest of the specimens were negative for HES-1. This result is seemingly different from the findings of the study executed by Cui and others in which TA and TSA polyps showed mixed positive and negative staining patterns for HES-1 (18). We observed no significant relationship between HES-1 expression and adenomatous polyp types, although there was a significant relationship between dysplasia of polyp types and HES-1 expression, which aligns with the association revealed by the previous studies (26).

Since the present study is cross-sectional, longitudinal follow-up data were unfeasible, and dysplastic changes or alterations in the prognosis of the disease remained unknown. In some patients with a colonoscopy report, the biopsy specimens were missed due to small sizes or inability to take it properly due to coagulation disorders in patients. On the other hand, the relationship between symptoms and endoscopic findings has not been analyzed in the current study due to limited resources.

In conclusion, we observed a significant relationship between HES-1 expression and both types of colorectal polyps and their locations. We also showed a significant relationship between the expression of HES-1 and dysplasia of polyps, which is consistent with the earlier study (35). HES-1 can be used as a potential marker for differentiating between SSA/P and hyperplastic polyps with high sensitivity and specificity, and our findings suggest that HES-1 expression holds promise for the future development in early diagnosis and more effective therapy for CRC that supports the idea of previous studies (36, 37).

CONCLUSION

More extensive research will help us to unfold different tumorigenesis mechanisms. Recommendations regarding the utilization of these markers in routine approaches to CRC require more detailed clinical and molecular studies with larger samples.

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AUTHORS' CONTRIBUTION:

BK, NZ, MGH, TM, AM, AGH conceived the study, performed data analysis, and wrote the manuscript, collected data and wrote the manuscript, interpreted the results and wrote the manuscript, designed the study, wrote, and edited the manuscript.

CONFLICT OF INTERESTS STATEMENT:

The authors have no conflicts of interest to declare related to this work.

DATA AVAILABILITY:

The dataset presented in the study is available at the request of the corresponding author during submission or after its publication.

ETHICAL APPROVAL:

The ethics committee of Shahid Beheshti University of Medical Sciences, Ilam, Iran, approved the study with the ID number IR.SBMU.RETECH.REC.1397.617.
https://ethics.research.ac.ir/ProposalCertificateEn.php?id=30026&Print=true&NoPrintHeader=true&NoPrintFooter=true&NoPrintPageBorder=true&LetterPrint=true

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REFRENCES:

1. Nourmohammadi H, Motaghi M, Soltany B. The Effects of Reflexology on Fatigue Severity of Patients with Cancer. *Asian Pac J Cancer Prev*. 2019;20(2):391-4.
2. Nourmohammadi H, Otaghi M, Salimi AH, Tarjoman A. Positive Effects of Cognitive Behavioral Therapy on Depression, Anxiety and Stress of Family Caregivers of Patients with Prostate Cancer: A Randomized Clinical Trial. *Asian Pac J Cancer Prev*. 2017;18(12):3207-12.
3. Borji M, Tarjoman A, Abdi A, Otaghi M. Efficacy of Implementing Home Care Using Eye Movement Desensitization and Reprocessing in Reducing Stress of Patients with Gastrointestinal Cancer. *Asian Pac J Cancer Prev.* 2019;20(7):1967-71.
4. Akaeva D, Maazova Z, Kaushanskaya L, Vavshko A, Yushko Z, Pyatakova A, et al. Dynamics of heart performance in patients with cervical cancer using neoadjuvant polychemotherapy. *J Med Pharm Chem Res*. 2025;7:1904-1916.
5. Sakinah J, Rohmatika AU, Lim V, Demircan T, Dewi FRP. Discovering the potential of bioactive compounds from curcuma aeruginosa as anti-ovarian cancer agent through in silico approach targeting TNFα. *J Med Pharm Chem Res*. 2025;7(8):1730-46.
6. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394-424.
7. Siahaan TCEO, Matulatan F, Budiman SP. Hartmann's anterior resection procedure in the management of colorectal cancer in an adolescent female- A rare clinical encounter. *J Med Pharm Chem Res*. 2025;7(4):591-7.
8. Jemal A, Center MM, DeSantis C, Ward EM. Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiol Biomarkers Prev*. 2010;19(8):1893-907.
9. Benett A, Goldblum JR, Odze RD. Inflammatory Disorders of the GI Tract. Surgical Pathology of the GI Tract, Liver, Biliary Tract and Pancreas E-Book. 2009:231.
10. Edwards BK, Ward E, Kohler BA, Eheman C, Zauber AG, Anderson RN, et al. Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. *Cancer*. 2010;116(3):544-73.
11. Yiu HY, Whittemore AS, Shibata A. Increasing colorectal cancer incidence rates in Japan. *Int J Cancer*. 2004;109(5):777-81.
12. Hossein Somi M, Mirinezhad K, Farhang S, Jazayeri E, Sani A, Seif-Farshadi M, et al. Gastrointestinal cancer occurrence in East Azarbaijan: a five year study from North Western Iran. *Asian Pac J Cancer Prev*. 2006;7(2):309-12.
13. Malekzadeh R, Bishehsari F, Mahdavinia M, Ansari R. Epidemiology and molecular genetics of colorectal cancer in iran: a review. *Arch Iran Med*. 2009;12(2):161-9.
14. Noffsinger AE. Serrated polyps and colorectal cancer: new pathway to malignancy. *Annu Rev Pathol*. 2009;4:343-64.
15. Gupta S, May FP, Kupfer SS, Murphy CC. Birth Cohort Colorectal Cancer (CRC): Implications for Research and Practice. *Clin Gastroenterol Hepatol*. 2024;22(3):455-69.e7.
16. Patel SG, Dominitz JA. Screening for Colorectal Cancer. *Ann Intern Med*. 2024;177(4):Itc49-itc64.
17. Levine JS, Ahnen DJ. Clinical practice. Adenomatous polyps of the colon. *N Engl J Med*. 2006;355(24):2551-7.
18. Cui M, Awadallah A, Liu W, Zhou L, Xin W. Loss of Hes1 Differentiates Sessile Serrated Adenoma/Polyp From Hyperplastic Polyp. *Am J Surg Pathol*. 2016;40(1):113-9.
19. Nussrat FL, Ali HH, Hussein HG, Al-Ukashi RJ. Immunohistochemical Expression of ki-67 and p53 in Colorectal Adenomas: A Clinicopathological Study. *Oman Med J*. 2011;26(4):229-34.
20. Sheikh RA, Min BH, Yasmeen S, Teplitz R, Tesluk H, et al. Correlation of Ki-67, p53, and Adnab-9 immunohistochemical staining and ploidy with clinical and histopathologic features of severely dysplastic colorectal adenomas. *Dig Dis Sci*. 2003;48(1):223-9.
21. Feder JN, Li L, Jan LY, Jan YN. Genomic cloning and chromosomal localization of HRY, the human homolog to the Drosophila segmentation gene, hairy. *Genomics*. 1994;20(1):56-61.
22. Kageyama R, Ohtsuka T, Kobayashi T. The Hes gene family: repressors and oscillators that orchestrate embryogenesis. *Development*. 2007;134(7):1243-51.
23. Kageyama R, Ohtsuka T. The Notch-Hes pathway in mammalian neural development. *Cell Res*. 1999;9(3):179-88.
24. Kageyama R, Ohtsuka T, Kobayashi T. Roles of Hes genes in neural development. *Dev Growth Differ*. 2008;50 Suppl 1:S97-103.
25. Sail V, Hadden MK. Identification of small molecule Hes1 modulators as potential anticancer chemotherapeutics. *Chem Biol Drug Des*. 2013;81(3):334-42.
26. Gao F, Zhang Y, Wang S, Liu Y, Zheng L, Yang J, et al. Hes1 is involved in the self-renewal and tumourigenicity of stem-like cancer cells in colon cancer. *Sci Rep*. 2014;4:3963.
27. Wu Y, Gong L, Xu J, Mou Y, Xu X, Qian Z. The clinicopathological significance of HES1 promoter hypomethylation in patients with colorectal cancer. *Onco Targets Ther*. 2017;10:5827-34.
28. Yuan R, Ke J, Sun L, He Z, Zou Y, He X, et al. HES1 promotes metastasis and predicts poor survival in patients with colorectal cancer. *Clin Exp Metastasis*. 2015;32(2):169-79.
29. Weng MT, Tsao PN, Lin HL, Tung CC, Change MC, Chang YT, et al. Hes1 Increases the Invasion Ability of Colorectal Cancer Cells via the STAT3-MMP14 Pathway. *PLoS One*. 2015;10(12):e0144322.
30. Ahadi M, Andrici J, Sioson L, Sheen A, Clarkson A, Gill AJ. Loss of Hes1 expression is associated with poor prognosis in colorectal adenocarcinoma. *Hum Pathol*. 2016;57:91-7.

31. Liu ZH, Dai XM, Du B. Hes1: a key role in stemness, metastasis and multidrug resistance. *Cancer Biol Ther*. 2015;16(3):353-9.
32. van Es JH, van Gijn ME, Riccio O, van den Born M, Vooijs M, Begthel H, et al. Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature*. 2005;435(7044):959-63.
33. Goldstein NS, Bhanot P, Odish E, Hunter S. Hyperplastic-like colon polyps that preceded microsatellite-unstable adenocarcinomas. *Am J Clin Pathol*. 2003;119(6):778-96.
34. Fujita K, Yamamoto H, Matsumoto T, Hirahashi M, Gushima M, Kishimoto J, et al. Sessile serrated adenoma with early neoplastic progression: a clinicopathologic and molecular study. *Am J Surg Pathol*. 2011;35(2):295-304.
35. Maeda T, Suzuki K, Togashi K, Nokubi M, Saito M, Tsujinaka S, et al. Sessile serrated adenoma shares similar genetic and epigenetic features with microsatellite unstable colon cancer in a location-dependent manner. *Exp Ther Med*. 2011;2(4):695-700.
36. Peignon G, Durand A, Cacheux W, Ayrault O, Terris B, Laurent-Puig P, et al. Complex interplay between β-catenin signalling and Notch effectors in intestinal tumorigenesis. *Gut*. 2011;60(2):166-76.
37. Ueo T, Imayoshi I, Kobayashi T, Ohtsuka T, Seno H, Nakase H, et al. The role of Hes genes in intestinal development, homeostasis and tumor formation. *Development*. 2012;139(6):1071-82.