A Comparative study of clinico-pathologic features and molecular alterations between serrated lesions and tubular adenoma of colon

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A Comparative study of clinico-pathologic features and molecular alterations between serrated lesions and tubular adenoma of colon

Directed by Professor Hyun-Soo Kim

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This certifies that the Master's Dissertation of

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ABSTRACT

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Background : Approximality 20-30% of CRC develop from serrated neoplasia pathway characterized by aberrant DNA methylation and BRAF mutation. Although, this serrated pathway including of either BRAF and KRAS mutations could be biologically heterogeneous as reflected in

sessile serrated adenomas (SSAs). Serrated polyps represent a heterogeneous group of polyps which are the precursor lesions to serrated pathway in CRC. Serrated lesions classified as hyperplastic polyp, sessile serrated adenoma/polyp (SSA/P) with or without cytological dysplasia, traditional serrated adenoma (TSA). SSA and TSA are premalignant lesions, but SSA is the principal serrated precursor of CRCs. Characterization of serrated polyp subtype in the field of clinical and epigenetic is still limited. **Purpose of the study :** Therefore, we tested the hypothesis whether BRAF V600E, LINE1, IGFBP7, hMLH1, and CD133 might be expressed in serrated lesion and tubular adenoma of colon and its expression may be correlated with serrated neoplasia pathway. **Methods :** We examined BRAF V600E mutation and methylation of LINE1, IGFBP7, hMLH1, and CD133 in 110 formalin fixed paraffin embedded (FFPE) tissue samples including 53 TSAs, 10 SSAs, and 47 tubular adenomas (TAs) by bisulfite conversion and pyrosequencing assay. **Results :** We found that BRAF V600E mutation was identified as 25 (47.2%), 5 (50%), and 0 (0%) in TSAs, SSAs and TAs, respectively. The frequency of methylation at LINE1, IGFBP7, hMLH1 and CD133 was 51 (96.2%), 20 (37.7%), 3 (5.7%) and 4 (7.5%) in TSAs compared to 10 (100%), 8 (80%), 0 (0%), and 3 (30%) in SSAs, and 46 (97.9%), 4 (8.5%), 1 (2.1%),

and 10 (21.3%) in TAs, respectively. These results evaluated that aberrant methylated genes could be detected frequently in serrated lesions and TAs of colon. **Conclusions :** This study elucidated that SSA is more hypermethylated compared to TA and TSA in LINE1 and IGFBP7. However hMLH1 and CD133 didn't different according to histological types. Also BRAF V600E mutation was frequently found in 25(47.5%) of TSAs and 5(50%) of SSAs compared to conventional tubular adenomas. Further would study with larger number of samples is needed to demonstrate the pathogenesis of serrated neoplasias, which is closely related with CIMP high, BRAF mutation and MSI.

Keywords: Sessile serrated adenoma, SSA, traditional serrated adenoma. TSA, tubular adenoma, TA, BRAF, LINE1, IGFBP7, hMLH1, CD133

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I. INTRODUCTION

The world-wide incidence of colorectal cancer (CRC) accounts about 1.2 million new cases per year and 40-50% of patients die within 5 years of diagnosis.[1-3] CRC develops through two main pathways. The most well-known pathway is adenocarcinoma pathway and its precursor lesions are tubular adenomas, tubulovillous adenomas, and villous adenomas.[4-6]

Another pathway is “serrated neoplasia pathway” which is described recently. This pathway suggests the progression of serrated polyps with specific histological characteristics, including saw-toothed infolding of the crypt epithelium, perhaps develop into CRC with microsatellite instability (MSI).[7, 8]

Thirty years ago serrated lesions were named as “hyperplastic polyp” (HP) and were understood no malignant potential. Since then, the serrated lesions have been established and the serrated polyps are the precursors of one-third of all CRCs.[9-11] Serrated lesions of the colorectal are classified pathologically according to the World Health Organization (WHO) criteria as hyperplastic polyps, sessile serrated adenoma/polyp (SSA/P) with or without cytological dysplasia, traditional serrated adenoma (TSA) (**Table 1**).[12]

Table 1. Pathological classification of serrated colorectal lesions recommended by the World Health Organization (2010)

Hyperplastic polyp
Sessile serrated adenoma/polyp with or without cytological dysplasia
Traditional serrated adenoma

The terms “sessile serrated adenoma” and “sessile serrated polyp” are considered synonymous

Recent studies have shown that serrated polyps are the precursors of CRCs developing through the serrated neoplasia pathway characterized by BRAF mutation, CpG island methylator phenotype (CIMP), with or without microsatellite instability (MSI). Either one or a combination of these lesions can be involved in the serial transition from normal epithelium to polyps, and to CRC.[13] The CpG island methylator phenotype (CIMP) is described the coordinate hypermethylation of multiple CpG dinucleotide clusters called CpG islands. These CpG islands often reside in gene promoter regions where aberrant DNA hypermethylation frequently correlates with silencing of the

downstream gene. The phenotype targets many hundreds of CpG islands; however, the specific gene promoters involved and whether the associated genes become silenced and play a role in the serrated neoplasia pathway. The epigenetic CIMP pathway is defined as the major mechanism driving the serrated pathway to CRC.[12] This pathway is based on abnormal promoter CpG island hypermethylation.[14-16] Methylation of CpG islands within promoter regions of genes is a normal way of reducing gene expression. More methylation means less expression, and if the gene being silenced is a tumor-suppressor gene, then loss of function may cause carcinogenesis. The extent of promoter CpG island hypermethylation in neoplasms varies considerably.[12] BRAF is a member of the RAF family of serine/threonine kinases which mediates cellular responses to growth signals through the RAS-RAF-MAP kinase pathway. BRAF mutations play a role in chromosomal instability pathway of CRC and have recently been found 5–15% of CRCs.[17] MSI pathway of CRC is characterized by the loss of mismatch repair gene function which leads DNA replication errors. Loss of mismatch repair usually occurs because of germline mutation of one of four mismatch-repair gene (*MSH2, MLH1, MSH6 or PMS2*). Recently, studies suggested that methylation of IGFBP7 is an important alteration in the serrated

neoplasia pathway and has correlation with MLH1 methylation, BRAF mutation, CIMP, and MSI in CRC. However, the pathological and epigenetic features of serrated polyps with methylated IGFBP7 are still largely unknown.[18] CD133 is a well-studied cancer stem cell marker, its function and prognostic significance are not established in CRC.[19] LINE-1 hypomethylation has showed a poor outcome in several types of human neoplasms, such as colon, stomach and ovarian cancer. [20, 21]

Previous studies reported that SSAs are mainly observed in the proximal colon and are associated with frequent BRAF mutation and CIMP that may be the precursor lesion of colorectal carcinogenesis with MSI. In contrast, TSAs are more commonly found in the distal colon and show frequent KRAS mutation.[7, 8, 11, 22-30] BRAF V600E mutation was rarely observed in TA but was higher frequently found in serrated polyps. Although some investigators have identified close association of BRAF mutation and promoter methylation, MSI in CRC. Among serrated polyps, BRAF V600E mutation occurred more frequently in SSA (75%) and mixed polyps (89%) compared with those of HP (19%) and SA (20%).[8, 17, 31-34]

Therefore, we tested the hypothesis whether BRAF V600E, LINE1, IGFBP7, hMLH1, and CD133 might be expressed in serrated lesion and tubular adenoma of colon and also its expression may be correlated with serrated neoplasia pathway. The study evaluated BRAF V600E mutation and methylation of LINE1, IGFBP7, hMLH1, and CD133 in these polyps by bisulfite conversion and pyrosequencing assay. Our data may useful to understand the serrated neoplasia pathway of CRC and clinical significance of promoter methylation.

II. MATERIALS AND METHODS

1. SAMPLE COLLECTION

We collected 110 formalin-fixed, paraffin-embedded (FFPE) tissue blocks of colorectal serrated lesions and tubular adenomas from the Department of Pathology, Yonsei university Wonju Severance Christian Hospital from 2005 to 2013. Fifty-three cases were TSAs, 47 were TAs and 10 were SSAs without cytological dysplasia. (shown Figure1.) Histological slides of all samples were reviewed by the gastrointestinal pathologist.

The patients comprised 66 males and 44 females and median age was 61.2 years (mean ± SD, 61.2±11.2). The “proximal” designation included the cecum, ascending colon, hepatic flexure and transverse colon. The “distal” colon included the descending, sigmoid colon and the rectum. Our purpose has been used to collect large polyps in this study.

2. ETHICS APPROVAL

The study has been approved by the Institutional Ethics Committee of Yonsei University Wonju Severance Christian Hospital (CR312041).

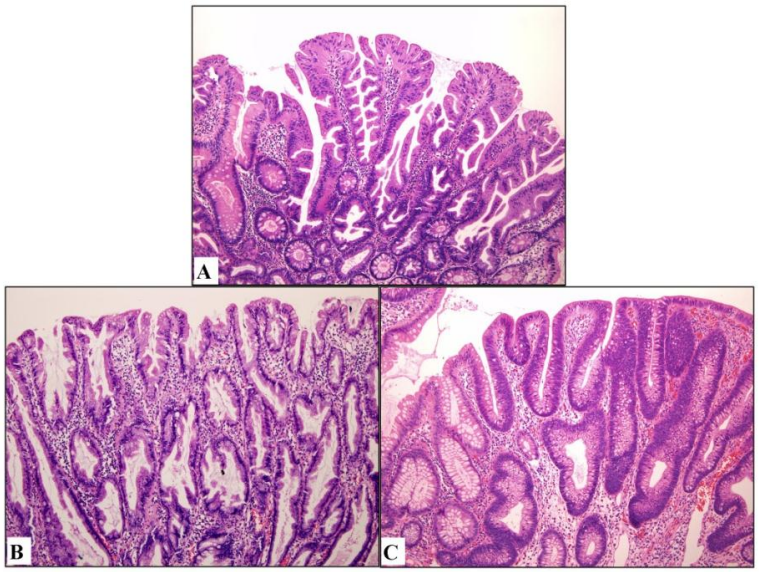


Figure 1. Histological images of serrated lesion and tubular adenoma of colon (H&E, x 100). **A**, Traditional serrated adenoma, which has prominent serration with presence of hypereosinophilic cytoplasm and nuclear stratification. **B,** Sessile serrated adenoma without cytological dysplasia. Prominent serration at all levels of crypt, crypt dilation, and branching with irregular distribution of goblet cells. **C,** Tubular adenoma, showing presence of pseudostratification with hyperchromatic nuclei.

3. DNA EXTRACTION AND BISULFITE CONVERSION

DNA extraction

Genomic DNA was extracted from FFPE tissue using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Tissue sections of 5 mm thickness were cut from paraffin blocks and 5 sections were placed into sterile microtubes. 1ml of xylene was added to each tube and vortexed for 10 seconds. Samples were centrifuged at 13,000 rpm at room temperature for 5 minutes and the supernatants were discarded. Residual xylene was removed by washing the samples three times with 1 ml of 100% ethanol. Then samples were vortexed and centrifuged at 13,000 rpm for 5 minutes. The pellets were incubated at 37 ℃ for 10 minutes. The pellet was resuspended with 180µm buffer ATL 20 µm and was added mixed proteinase K solution and mixed by vortexing. Mixture was incubated at 56 ℃ for 1 hour and continued again at 90 ℃ for 1hour. After incubation, briefly centrifuged for 10 seconds and were added 200 µm buffer AL, 200 µm 100% ethanol to each samples and mixed by vortexing. Then the mixture was transferred to the QIAmp MinElute column and was centrifuged at 8,000 rpm for 1 minute.

Sample mixture were placed in new 2 ml collection tube and were discarded the filtrate. The QIAmp MinElute column was then washed with 500 µm of buffer AW1 and was centrifuged at 8,000 rpm for 1 minute. The supernatant was discarded and recovery procedure was repeated with a new 2 ml collection tube. 500 µm buffer AW2 was then added to the column, and the column was centrifuged at 8,000 rpm at 1 minute. The supernatant was discarded and was placed in a new 2 ml collection tube and centrifuged at 15,000 rpm for 3 minutes to dry the membrane completely. Placed the QIAmp MinElute column in a clean 1.5 ml microcentrifuge tube. DNA in the QIAmp MinElute column was eluted by the addition of 30 µm of buffer ATE, followed 5 minutes incubation at room temperature and centrifugation at 14,000 rpm for 1 minute. The eluted DNA (30 µm volume) was then used as a template for PCR and Pyrosequencing.

Bisulfite modification of the genomic DNA was carried out using an EpiTect Bisulfite kit (Qiagen, Hilden, Germany) following the manufacture's protocol. This modification converts unmethylated cytosine to uracil and leaves 5-methyl cytosine unchanged.

Bisulfite reaction contained the following; 18 µm(500 ng) DNA solution, 2 µm RNase-free water, 85 µm Bisulfite Mixture, and 35 µm DNA Protect Buffer. PCR tubes were closed and mixed bisulfite reactions thoroughly. Then we performed the bisulfite DNA conversion using a thermal cycler following protocol and this step were incubated for 6 hours. Briefly, centrifuged and transferred to new 1.5 ml microcentrifuge tubes. Buffer BL/carrier RNA (560 µl) was added to the 140 µl of bisulfite-converted DNA sample and the mixture was transferred to EpiTect spin column and centrifuged at maximum speed for 1 minute. The supernatant was discarded and recovery procedure was repeated with a new 2 ml spin column. Buffer BW (500 µl) was then added, and after extensive mixing, the mixture was loaded onto the provided spin columns in consecutive 630-µl aliquots. After each loading, the columns were centrifuged at maximum speed for 1 minute. The supernatant was discarded and was placed the spin columns back. Buffer BD (500 µm) was added to each spin column and incubated at room temperature for 15 minutes followed by centrifugation at maximum speed for 1 minute. The supernatant was discarded and was placed the spin columns back. The spin column was then washed with 500 µl of buffer BW, followed by centrifugation at maximum speed (15,000 rpm) for 1 minute. The

supernatant was discarded and was placed the spin columns back. This step was repeated. DNA in the spin column was eluted by the addition of 20 µl of buffer EB, followed by a 1 minute incubation at room temperature and centrifugation at 12,000 rpm for 1 minute. The eluted DNA (20 µl volume) was then used for Pyrosequencing analysis and MethyLight analysis.

4. PCR AMPLIFICATION AND GEL ELECTROPHORESIS

PCR amplification was performed by HotStarTaq Plus Master Mix kit (Qiagen, Hilden, Germany) and random primers (Bioneer) according to manufacturer's instructions with some modifications. We used a PCR mixture containing 12.5 µl 2X Hot Star Taq Plus Master Mix, 0.5 µl Forward primer, 0.5 µl Reverse primer , 2.5 µl 10X Corallood Concentrate, 7 µl RNase-free water, 2 µl Template DNA. The PCR condition included initial denaturation at 94 ℃ for 5 minutes, annealing at 54-60 ℃ for 30 seconds, chain extension at 72 ℃ for 1minute, with a final extension at 72 ℃ 10 minutes for 45 cycles and finally 4 ℃. The reaction mixture was stored at 4 ℃, until it was loaded onto the gel. The amplified products were separated

on 2% agarose gels, stained with ethidium bromide (1 mg/ml), and visualized using a transilluminating UV chamber (Alpha Innotech, CA, USA).

Primer sequences and conditions for PCR and pyrosequencing assay are shown in **Table 2.**

5. PYROSEQUENCING ASSAY

Pyrosequencing was carried out using PSQ HS 24 Gold single-nucleotide polymorphism reagents on a PSQ HS 24 pyrosequencing machine (Biotage, Uppsala, Sweden). The results were analyzed usingQ-CpGsoftware (Qiagen). All primers were designed on Pyrosequencing Assay Design (Qiagen, Hilden, Germany).

Pyrosequencing was then performed on the PCR products with reagents from the PyroGold Reagents kit (Qiagen, Hilden,Germany), 1 × annealing

buffer (200 mmol/L Tris acetate, 50 mmol/L magnesium acetate), binding buffer at ph 7.6 (10 mmol/L Tris-HCI, 2 M NaCL, 1 mmol/L EDTA, 1 ml/l Tween 20), 3µl Streptavidin Sepharose High Performance beads and 15 µM pyrosequencing primer. The nucleotide dispensation orders were: LINE1, TTYGTGGTGYGTYGTT, CD133, YGAGGTTATTTTTTYGYGTTYGT, hMLH1, YGTGAGTAYGAGGTATTGAGGTGATT, IGFBP7, and TYGAYGTTAGTAGGAGYGYGYGYG, as previously reported.[35-37] The amount of C relative to the sum of the amounts of C and T at each CpG site was calculated as a percentage (i.e., 0-100). The average of the relative amounts of C in the CpG sites was used as the overall methylation level of each gene in colon polyps.

BRAF MUTATION ANALYSIS

BRAF V600E mutation was examined by Pyrosequencing using Pyrokit (Qiagen) according to the manufacturer's instructions. For detection of the BRAF mutation, genomic DNA obtained from FFPE samples was amplified using the following primers of BRAF gene (5'-GAAGACCTCACAGTAAAAATAG and 5'ATAGCCTCAATTCTTACCATCC), as previously reported[38]

Table 2. Primer sequences in PCR and Pyrosequencing assay

Gene locus	Type	Primer sequence 5'-3'	Annealing temperature (℃)	Length (bp)
BRAF	F	GAAGACCTCACAGTAAAAATAG	51	122
	R	ATAGCCTCAATTCTTACCATCC		
	S	GATTTTGGTCTAGCTACA		
		G**T**/**A**GAAATCTCGA		
LINE1	F	TTTTGAGTTAGGTGTGGGATATA	51	310
	R	AAAATCAAAAAATTCCCTTTC		
	S	AGTTAGGTGTGGGATATAGT		
		TT**Y**GTGGTG**Y**GT**Y**GTT		
IGFBP7	F	AGGGTTYGGGGTAGGGGATTGGGGAT	60	208
	R	AAAACCACACCCCRAAACRATAAAAACAC		
	S	YGGGTGTTYGTTTATTTT		
		T**Y**GA**Y**GTTAGTAGGAG**Y**G**Y**G**Y**G**Y**G		
hMLH1	F	TTGGTATTTAAGTTGTTTAATTAATAGTTG	56	119
	R	AAAATACCTTCAACCAATCACCTC		
	S	AGTTATAGTTGAAGGAAGAA		
		YGTGAGTA**Y**GAGGTATTGAGGTGATT		
CD133	F	GGAGTAGGGATATGGGGGTATAAA	55	163
	R	AAACACCCCAATTCTCCATCT		
	S	GGGATATGGGGGTATAAAG		
		YGAGGTTATTTTTT**Y**G**Y**GTT**Y**GT		

6. STATISTICAL ANALYSIS

All statistical analysis was performed using PASW (version 20.0) (SPSS Inc., Chicago, IL, USA). Differences between the groups were evaluated using the one way ANOVA, Chi-square test and Fisher's exact test, respectively. Statistical significance tests were two-tailed, and $p < 0.05$ was considered statistically significant.

III. RESULTS

1. Clinical findings

The clinical features of all patients are shown in **Table 3.** In this study, 66 male and 44 female patients were included. The median age was 61.2 years (61.2 ±11.2). There were no significant differences in the age or gender of the patients in this study. We identified that 33 TSAs (62.3%) were located in distal colon, 35 TAs (74.5%) were occurred in the proximal colon, and 10 SSAs (100%) were found in the proximal colon; thus, it was statistically significant *(P=0.001*) . The mean size of TSAs, SSAs, TAs was 1.3±0.4cm (0.5-2.5cm), 1±0.2cm (0.5-1.2cm), TAs 1.2±0.5cm (0.3-2.5cm), respectively. There is a statistically significant difference in the location of polyps *(*P=0.000)* .

Table 3. Clinical features of patients with TA, TSA and SSA

Features	All polyps	P value	TA n (%)	TSA n (%)	SSA n (%)
No of patients	110		47	53	10
Age (Mean±SD)	61.2±11.2	0.392	62.4±10.8	60.9±11.1	57.1±14.1
Size (Mean±SD, mm)	12.2±4.5	0.052	11.6±4.9	13.2±4.1	9.9±2.1
Sex					
Male	66 (60)	0.146	33 (70.2)	27 (50.9)	6 (60)
Female	44 (40)		14 (29.8)	26 (49.1)	4 (40)
Location					
Proximal colon	65 (59.1)	0.000*	35 (74.5)	20 (37.7)	10 (100)
Distal colon	45 (40.9)		12 (25.5)	33 (62.3)	0 (0)

TA-Tubular adenoma , TSA-Traditional serrated adenoma, SSA-Sessile serrated adenoma,

*$P < 0.05$; statistically significant

2. Prevalence of BRAF mutation

BRAF V600E mutation was identified 25 (47.2%) and 5 (50%) in TSAs SSAs, respectively. However, none of 47 TAs detected BRAF mutation (**Table 4**).

3. Methylation status of LINE1, IGFBP7, hMLH1, CD133 in TAs, TSAs and SSAs

The methylation status of TAs, TSAs and SSAs are shown in **Table 4** . We used pre-established and validated criteria for LINE1 with percent methylated relative (PMR) ≥ 60 for this gene were considered to have hypermethylated. But, if PMR < 60 with LINE1 were hypomethylated. For IGFBP7 samples with PMR ≥ 20 were considered to have hypermethylated and PMR < 20 were defined as hypomethylated. For hMLH1 and CD133 samples with PMR ≥ 10 were considered to have hypermethylated and PMR < 10 were defined as hypomethylated.(shown Table 4)[18] The frequency of methylation at LINE1, IGFBP7, hMLH1 and CD133 was 51 (96.2%), 20 (37.7%), 3 (5.7%) and 4 (7.5%) respectively, in TSAs compared to 10 (100%), 8 (80%), 0 (0%), 3 (30%) in SSAs and 46 (97.9%), 4 (8.5%), 1 (2.1%), 10 (21.3%) in TAs. The frequency of methylation of LINE1 was hypermethylation in all these polyps *(P=0.755).* Although higher frequency of methylation of IGFBP7 in was largely due to a higher methylation frequency in SSAs than in TSAs and TAs 8 (80%) and 20 (37.7) versus 4 (8.5%), respectively *(*P=0.000).* hMLH1 was also didn't methylated in

SSAs compared to TSAs and TAs, 0(0%), 3 (5.7%), 1 (2.1%), respectively *(P=0.521)*. Moreover, the hypermethylation of CD133 in TAs, TSAs and SSAs were occurred in 6 (14%), 5 (9.4%) and 3 (30%), respectively *(P=0.208)*. There were not found a statistically significant difference.

Table 4. Correlations between clinical features and genetic and epigenetic changes in serrated lesions and tubular adenoma

Histology	TA	TSA	SSA	P value
BRAF mutation				0.000*
+	0 (0)	25 (47.2)	5 (50)	
–	47 (100)	28 (52.8)	5 (50)	
LINE1 (mean±SD,%)	65.7±3.2	68.1±4	74.8±2.1	0.755
≥60	46 (97.9)	51 (96.2)	10 (100)	
<60	1 (2.1)	2 (3.8)	0 (0)	
IGFBP7 (mean±SD,%)	14.5±9.2	23.1±20.1	34.9±15.9	0.000*
≥20	8 (17)	20 (37.7)	8 (80)	
<20	39 (83)	33 (62.3)	2 (20)	
hMLH1 (mean±SD,%)	5.6±1.7	6.4±2.6	6.9±1.1	0.521
≥10	1 (2.1)	3 (5.7)	0 (0)	
<10	46 (97.9)	50 (94.3)	10 (10)	
CD133 (mean±SD,%)	7.9±3.0	7.3±6.0	9.0±1.4	0.208
≥10	6 (14)	5 (9.4)	3 (30)	
<10	37 (86)	48 (90.6)	7 (70)	

TA-Tubular adenoma , TSA -Traditional serrated adenoma, SSA-Sessile serrated adenoma,

*$P < 0.05$; statistically significant

Figure 2. Methylation patterns of LINE1 in TAs, TSAs, and SSAs *(P=0.755).*

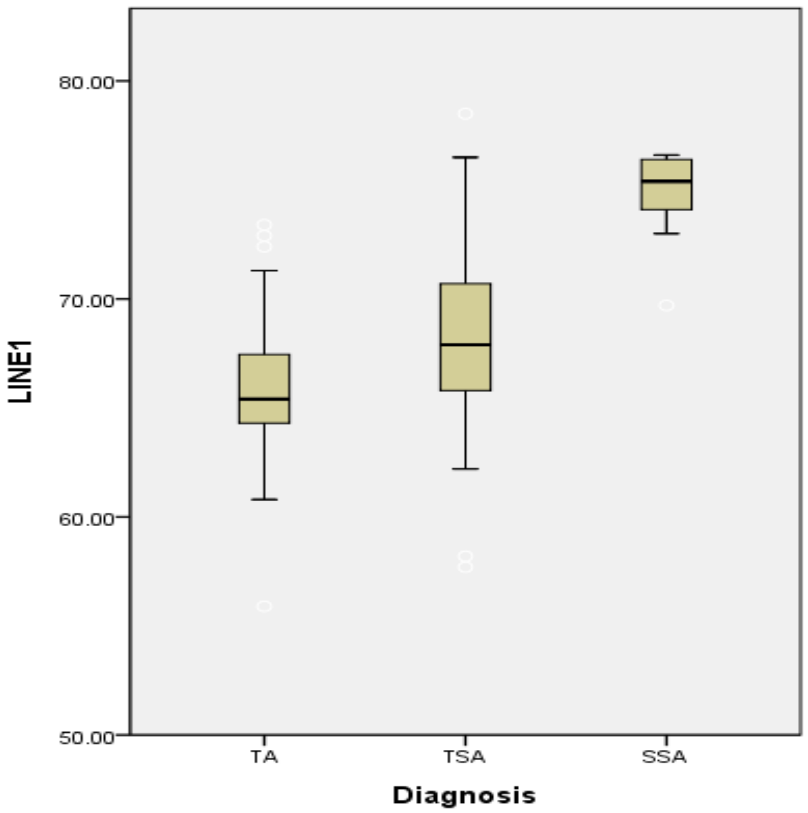


Figure 3. Methylation patterns of IGFBP7 in TAs, TSAs, and SSAs ***(*P=0.000).***

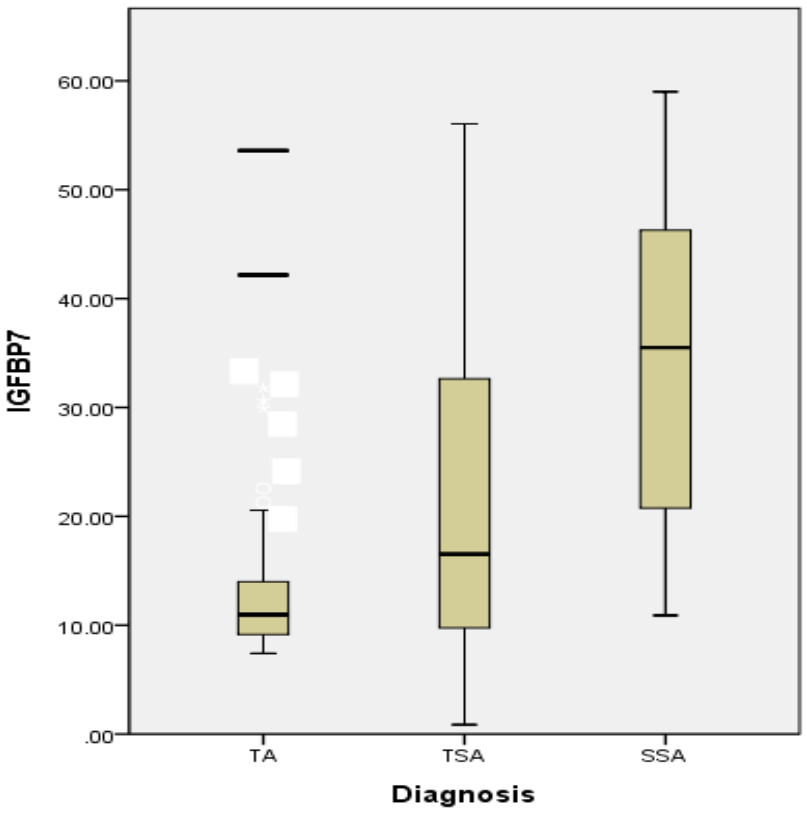


Figure 4. Methylation patterns of hMLH1 in TAs, TSAs, and SSAs *(P=0.521)*.

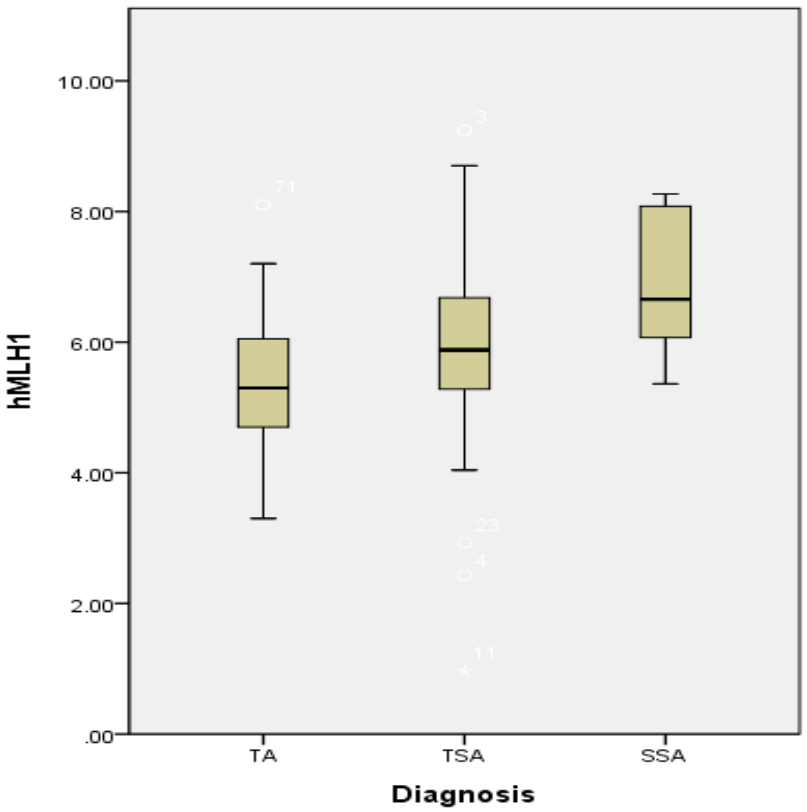
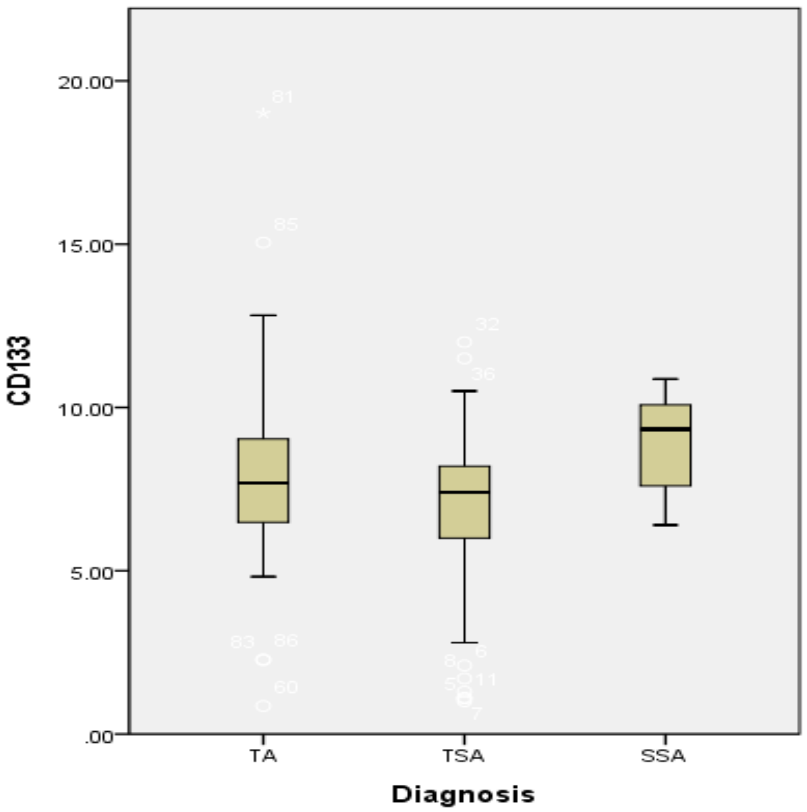


Figure 5. Methylation patterns of CD133 in TAs, TSAs, and SSAs
(P=0.208)



IV. DISCUSSION

In this study, we investigated epigenetic alterations in serrated lesions and tubular adenomas of colon, assessing by promoter gene methylation of LINE1, IGFBP7, hMLH1, CD133 and BRAF mutation. We analyzed these results with clinical and histological features and compared to TSAs, SSAs and TAs. The mean age and gender of patients within serrated lesions (TSAs, SSAs) and tubular adenomas were not found significant difference. Our study supported by some of previous studies that showed no association with age and gender of patients with serrated polyps.[39, 40] We identified that 33 TSAs (62.3%) were located in distal colon, 35 TAs (74.5%) were located in the proximal colon, and 10 SSAs (100%) were found in the proximal colon; and it was found to be statistically significant (*P=0.001*). The mean size of TSAs, SSAs, TAs were 1.3±0.4 cm, 1±0.2 cm, 1.2±0.5 cm, respectively. These findings are confirmed by serrated lesions appear larger polyps, SSAs are predominantly located in the proximal colon and TSAs and more likely to be located in the distal colon. TA often occurred in the proximal colon, which was similar with previous studies.[22, 41, 42]

The Mitogen Activated Protein kinase (MAPK) signaling pathway is generally altered in CRC and precursor lesions by oncogenic mutation of either the BRAF or KRAS genes. These mutations are mutually exclusive and revealed a striking specificity for serrated polyp subtype. [41] BRAF V600E mutation is an early event in the "serrated neoplasia pathway".[2] Rare occurrences of BRAF V600E mutation in adenomas and high frequent BRAF V600E mutations in serrated polyps was reported previously. Although in some studies on SSA and TSA, BRAF mutation was observed in 62.1% to 90% and 27 to 55%, respectively, but not in TAs. [23, 43-45] In our study, BRAF V600E mutation was observed in 5 (50%) of SSAs, and 25 (47.2%) of TSA, respectively. However, it was not found in tubular adenomas. The recent report of the BRAF V600E mutation in serrated lesions and TAs indicated that BRAF V600E mutation occurs very early in CRCs and could be a specific marker of both serrated polyps and serrated pathway.[43] CpG island methylation is an epigenetic process commonly involved in silencing of gene transcription leading to development of cancer [14]. The present study was carried out to clarify CpG island promoter methylation in serrated lesions and TAs. We used four tumor related genes (LINE1, IGFBP7, hMLH1, CD133) of CRCs and didn't observe any

significant differences between serrated lesions and TAs. Our study identified that new finding for LINE1 gene involved in serrated lesions and TAs. We observed that higher methylation of LINE1 was seen in 51 (96.2%), 10 (100%), and 46 (97.9%) for TSAs, SSAs and TAs, respectively. Although, methylation of IGFBP7 was observed as 20 (37.7%), 8 (80%) and 8 (17%) in TSAs, SSAs and TAs, respectively. In 2012, Eisuke Kaji et al [18] proposed, that inactivation of IGFBP7 is an early event in colorectal tumor genesis as IGFBP7 was shown to be methylated not only in CRC but in colorectal adenoma, while MLH1 methylation or MSI is rare in adenoma. IGFBP7 methylation plays a key role in the serrated pathway and to occur during the course from serrated polyp to cancer with MSI. These findings suggest that methylation of LINE1 and IGFBP7 genes may contribute to the progression of the polyps. We found little methylation of hMLH1 and CD133. The reason could be that mismatch repair (MMR) protein and CD133 was rare in serrated lesions and polyps and maybe to occur a late alteration that leads to dysplasia through polyps to CRCs. [46, 47] In human studies, ethical and practical barriers may make it difficult or impossible to collect specimens from the target tissue. However, our study sample size was not sufficient to fully conclude methylation status in serrated lesions and TAs.

V. CONCLUSION

In conclusion, this study elucidated that SSA is more hypermethylated compared to TA and TSA in LINE1 and IGFBP7. However hMLH1 and CD133 didn't different according to histological types. Also BRAF V600E mutation was frequently found in 25(47.5%) of TSAs and 5(50%) of SSAs compared to conventional tubular adenomas. Further would study with larger number of samples is needed to demonstrate the pathogenesis of serrated neoplasias, which is closely related with CIMP high, BRAF mutation and MSI.

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국문초록

대장의 톱니모양 병변들하고 관모양 샘종 사이의 임상병리학적 특징과 분자생물학적 변화의 비교 연구

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대장암의 약 20-30%는 비정상적인 DNA 메틸화와 BRAF 돌연변이 특징으로 톱니폴립 암화과정(serrated neoplastic pathway)에서 발생되고 있습니다. 실질적으로, BRAF 와 KRAS 돌연변이를 포함한 톱니폴립 암화과정이 정착 톱니 모양 선종들(SSAs)을 반영할 수 있습니다. 톱니폴립은 대장암의

톱니폴립 암화과정에 전구체 병변 폴립을 대변합니다. 톱니 모양의 병변은 크게 증식성 폴립, 무경성 톱니샘종/폴립 그리고 이형성 무경성 톱니 샘종/ 폴립과 전통 톱니 샘종으로 분류됩니다. 정착 톱니 모양 용종/ 선종와 기존의 톱니 모양 선종은 전암병변이지만, 정착 톱니 모양 용종/선종은 대장암의 중요 톱니 모양의 전구체입니다. 임상 및 후생 유전학적 분야에서의 톱니 폴립 하위유형의 특성에 대한 연구는 아직 제한적입니다.

따라서, 본 연구는 BRAF V600E, LINE1, IGFBP7, hMLH1 및 CD133 유전자들은 톱니 폴립의 병변 및 관상 선종에서(TA) 표현될 수 있으며 그 표현이 톱니폴립 암화과정과 상관관계가 있는지에 대해 연구하였습니다. 모두 110 포르말린 고정 파라핀 조직 샘플은 53 개의 전통 톱니 샘종, 10 개의 무경성 톱니 샘종/ 폴립, 47 개의 관상 선종들의 샘플에 대해 아황산 수소 변환과 pyrosequencing 기법을 이용하여 BRAF V600E 돌연변 및 LINE1, IGFBP7, hMLH1, CD133 의 메틸화에 대해 분석하였습니다. BRAF V600E 돌연변이는 전통 톱니 샘종, 무경성 톱니 샘종/폴립, 관상 선종에서

각각 25(47.2%), 5(50%), 0(0%)으로 분석되었습니다. LINE1, IGFBP7, hMLH1 과 CD133 의 빈도는 전통 톱니 샘종에서는 51(96.2%), 20(37.7%), 3(5.7%), 4(10%)로, 무경성 톱니 샘종/ 폴립에서는 10(100%), 8(80%), 0(0%), 3(30%)로, 관상 선종에서는 46(97.9%), 4(8.5%), 1(2.1%), 10(21.3%)로 각각 측정되었습니다. 위 결과는 비정상적인 메틸화 유전자는 대장의 톱니 폴립 병변과 관상 선종에서 발견된 것을 확인하였습니다.

결론적으로, BRAF V600E 돌연변이는 아마 톱니폴립 암화과정에 특이적인 마커가 될 수 있으며, 대장암 발암 과정의 유용한 자료가 될 것으로 생각됩니다.

핵심 단어: 정착 톱니 모양 선종, SSA, 기존의 톱니 모양 건종, TSA, 관상 선종, TA, BRAF, LINE1, IGFBP7, hMLH1, CD133.