

Evaluation of the Expression of the Inhibitor of Apoptosis Protein Family and Human Telomerase Reverse Transcriptase in Patients With Advanced Colorectal Adenoma

ORIGINAL
ARTICLE

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Background: It has been reported that the expression of the inhibitor of apoptosis protein (IAP) family increases in patients with colon cancer. We evaluated the expression of the IAP family and human telomerase reverse transcriptase (hTERT) in normal colon mucosa from patients with advanced colorectal adenoma and investigated their features according to characteristics of advanced colorectal adenoma.

Methods: While resections of polyps were performed in patients (n = 80) diagnosed with advanced colorectal adenoma or carcinoma in situ, additional normal tissues were obtained from the sigmoid colon. In healthy patients (n = 16), blind biopsies were performed on the sigmoid colon. The expression of the IAP family, including survivin, XIAP, cIAP1, and cIAP2, and hTERT, were analyzed by real-time PCR in both groups.

Results: A total of 80 advanced colorectal adenoma patients (71.3% male, mean age of 60.4 years) and 16 control patients were enrolled in this study. The mean ranking of cIAP2 was higher in the control group (68.88 vs. 44.43, $P = 0.001$). The expression levels of hTERT, survivin, XIAP, and cIAP from both groups showed no differences. The expression of survivin, XIAP, cIAP1, cIAP2, and hTERT depending on certain factors of advanced adenoma, including the number (two or fewer vs. three or more), size (smaller than 1 cm vs. larger than 1 cm), grade of dysplasia (low grade adenoma vs. high grade adenoma), pathology (tubular adenoma vs. villous adenoma), and presence of endometrial intraepithelial neoplasms, showed no significant correlations in the Mann-Whitney U-test.

Conclusions: The expression of the IAP family and hTERT, except cIAP2, in the normal mucosa of patients with advanced colorectal adenoma were not different from those of the control group. There were no differences in the IAP family and hTERT according to the characteristics of advanced adenoma.

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Key Words: Colonic neoplasm, Inhibitor of apoptosis proteins, Telomerase

INTRODUCTION

Colorectal cancer (CRC) is the fourth most common cause of cancer-related deaths.¹ The apoptotic and anti-apoptotic signaling pathways are important in regulating carcinogenesis and determining cancer progression and prognosis. Molecules associated

with apoptosis have significance as cancer diagnostic markers and therapeutic targets. The inhibitor of apoptosis protein (IAP) family includes several important molecules involved in apoptosis that might represent such targets. There is increasing evidence that the IAP family members are essential for anti-apoptotic signal transduction and that the expression of IAPs

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increases, leading to poor colon cancer outcome and prognosis and to the drug resistance of tumors.^{2,4} X-linked IAP (XIAP), cIAP1, cIAP2, and survivin are prognostic markers of colorectal cancer, and survivin and cIAP2 are also used to predict the efficacy of anticancer treatments in CRC patients.⁵

CRC also undergoes a multi-step process, which is associated with genetic alterations.⁶ Human telomerase reverse transcriptase (hTERT) stabilizes chromosomal ends by extending the telomeric repeat sequences and plays an important role in cell survival, development and the proper functioning of various organs.⁷ hTERT activity is inhibited in normal somatic tissues, but hTERT expression is increased in most human tumors.⁸ Telomerase reactivation in the cell is associated with carcinogenesis and is an important step in the immortalization process of tumors.⁹

It is not known whether the expression of major IAPs or hTERT is increased in normal colon tissue of patients with advanced adenoma or carcinoma in situ. In this study, we evaluated the factors associated with apoptosis and proliferation in the normal colon mucosa of patients with advanced adenoma or carcinoma in situ compared to normal subjects and the expression levels of these factors according to the characteristics of advanced adenoma.

MATERIALS AND METHODS

1. Study subjects

This is a prospective study conducted at the Seoul National University Bundang Hospital, Gyeonggi-do, Korea. To be included in the study, subjects were required to meet the following criteria: (1) Patients with pathologically-proven of advanced colorectal adenoma (3 or more adenoma, larger than 1 cm adenoma, villous adenoma, high grade dysplasia) or colonic adenoma in situ. (2) Patients receiving a colonoscopy for the purpose of routine screening or irritable bowel syndrome. Patients with a history of previous CRC, including hereditary CRC, colon resection, and known inflammatory bowel disease (Crohn's disease or ulcerative colitis), 85 years or older or high risk of bleeding were excluded.

In the 80 patients diagnosed with advanced adenoma or carcinoma in situ, normal tissues were collected at sigmoid colon about 20 cm above anal verge during the polypectomy procedure. Random biopsies were also performed on an AV 20 cm sigmoid colon in 16 patients who underwent a colonoscopy for the purpose of medical check-up or suspected irritable bowel syndrome.

The expression levels of the major IAPs, such as XIAP, cIAP1, cIAP2, survivin and hTERT, in both groups were analyzed by real-time PCR. In addition, we analyzed whether the expression of these factors varies according to the characteristics of the advanced adenomas (number, size, grade of dysplasia, and pathology). The study protocol was approved by the Institutional Review Board of SNUH (IRB No. B-1308/215-008).

2. Quantitative real-time PCR

The rotor gene system (Corbett Research, Sydney, Australia) was used to run quantitative real-time polymerase chain reaction. The absolute quantification assay was chosen to analyze hTERT expression. PCR was performed using a total reaction volume of 20 μ L containing 1x TaqMan universal master mix with AmpErase uracil N-glycosylase (to prevent the re-amplification of carry-over PCR products; Applied Biosystems, Foster City, CA, USA), 600 nM of each primer, 200 nM TaqMan probe, and 1 μ L of unknown cDNA or 2 μ L of the standard template. All of the samples (unknown or standard) were run in duplicate and accompanied by a non-template control. Thermal cycling conditions included 2 minutes at 50°C and 10 minutes at 95°C, followed by 45 cycles at 95°C for 15 seconds and 60°C for 60 minutes.

The primers for survivin were as follows: sense primer 5'-ATGGGTGCCCCGACGTTG-3'; reverse primer 5'-AGAGCCTCAATCCATGG-3'. The XIAP primers were as follows: sense primer 5'-GGCCATCTGAGACACATGCAG-3'; reverse primer 5'-GCATTCACTAGATCTGCAACC-3'. The cIAP1 primers were as follows: sense primer 5'-GCCTGATGCTGGATAACTGG-3'; reverse primer 5'-GGC-GACAGAAAAGTCAATGG-3'. The cIAP2 primers were as follows: sense primer 5'-GGACAGGAGTTCATCCGTCAA-3'; reverse primer 5'-GGGCTGTCTGATGTGGATAGC-3'. The cIAP2 primers were as follows: sense primer 5'-GCATTGGAATCAGACAGCAC-3'; reverse primer 5'-CCACGACGTAGTCCATGTTC-3'.

3. Statistical analysis

In each group, continuous variables such as age were compared using an ANOVA, and categorical variables, such as gender were compared using a crossover analysis (chi-squared crossover). The Mann-Whitney U-test was used to analyze the expression of IAPs and hTERT in both groups. All statistical tests were performed with the program IBM SPSS ver. 22.0 (IBM Co., Armonk, NY, USA). *P* values of < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics of the study populations were shown

in Table 1. There were no significant differences between the two groups with regards to sex and age ($P = 0.134$). The mean age was 60.4 years, and males comprised 71.3% of the population. Three or more adenomas were found in 46.3% of the patients, and adenomas larger than 1 cm were observed in 33.8% of the patients. High grade adenoma accounted for 15.0% of the cases, and villous adenoma accounted for 23.8%. Early CRC was found in 11.3% of the patients.

The Mann-Whitney U-test was used to determine whether there were significant differences in the expression of hTERT,

Table 1. Baseline clinicopathologic characteristics of 80 patients with advanced colorectal adenoma

Characteristic	Value
Age (yr)	60.4 ± 11.4
Sex	
Male	57 (71.3)
Female	23 (28.8)
Multiple polyps	
Two or less	43 (53.8)
Three or more	37 (46.3)
Size	
Smaller than 1 cm	53 (66.3)
larger than 1 cm	27 (33.8)
Grade of adenoma	
Low grade	68 (85.0)
High grade	12 (15.0)
Pathology	
Tubular adenoma	61 (76.3)
Villous adenoma	19 (23.8)
IEN	
IEN	9 (11.3)
Other than	71 (88.8)
SSA	
SSA	9 (11.3)
No SSA	71 (88.8)

Values are presented as the mean ± SD or number (%). The sum of the percentages does not equal 100% because of rounding. IEN, intraepithelial neoplasia; SSA, sessile serrated adenoma.

Table 2. Expression of IAPs and hTERT in the adenoma and control groups

Variable	Adenoma group (n = 80)	Control group (n = 16)	Pvalue
hTERT	39.74 ± 122.64	5.02 ± 9.64	0.159
Survivin	13.83 ± 39.96	7.82 ± 20.05	0.469
XIAP	7.80 ± 29.43	7.09 ± 11.92	0.101
cIAP1	22.08 ± 63.01	16.90 ± 6.72	0.258
cIAP2	1.30 ± 3.65	1.70 ± 1.55	0.001

Values are presented as mean ± SD. IAP, inhibitor of apoptosis proteins; hTERT, human telomerase reverse transcriptase.

survivin, XIAP, cIAP, or cIAP2 between the experimental group and the normal control group. There was a significant difference only in cIAP2 between the two groups, and the average ranking was rather higher in the normal control group ($P = 0.001$) (Table 2).

When assessing the expression of survivin, XIAP, cIAP1, cIAP2, and hTERT in terms of the characteristics of advanced adenoma, such as number (two or fewer vs. three or more), size (less than 1 cm vs. more than 1 cm), grade of dysplasia (low grade adenoma vs. high grade adenoma), pathology (tubular adenoma vs. villous adenoma), and presence of endometrial intraepithelial neoplasm, we found no significant correlations using the Mann-Whitney U-test (Table 3-5).

DISCUSSION

Resistance to apoptotic stimuli is a hallmark feature of various cancers. Classical apoptosis is carried out through caspases and cysteine proteases, which induce several biochemical and molecular changes, including DNA fragmentation, ultimately leading to cell death.¹⁰ Overexpression of the IAPs is one of the mechanisms by which tumor cells acquire resistance to apoptosis.¹¹ IAPs represent a large family of endogenous caspase

Table 3. Expression of IAPs and hTERT according to the number of polyps

Variable	No. of polyp		Pvalue
	Two or less	Three or more	
hTERT	32.05 ± 88.03	47.42 ± 150.33	0.718
Survivin	14.77 ± 44.44	12.91 ± 35.61	0.737
XIAP	4.91 ± 9.85	10.69 ± 40.50	0.959
cIAP1	35.86 ± 85.48	8.29 ± 18.61	0.579
cIAP2	1.35 ± 4.52	1.24 ± 2.58	0.336

Values are presented as mean ± SD. IAPs, inhibitor of apoptosis proteins; hTERT, human telomerase reverse transcriptase.

Table 4. Expression of IAPs and hTERT according to the size of polyps

Variable	Size of polyp		Pvalue
	Smaller than 1 cm	Larger than 1 cm	
hTERT	56.38 ± 148.15	7.07 ± 13.88	0.430
Survivin	15.58 ± 44.37	10.59 ± 30.64	0.128
XIAP	10.84 ± 35.76	1.83 ± 4.41	0.108
cIAP1	25.82 ± 74.26	14.74 ± 30.83	0.112
cIAP2	1.73 ± 4.42	0.45 ± 0.64	0.840

Values are presented as mean ± SD. IAPs, inhibitor of apoptosis proteins; hTERT, human telomerase reverse transcriptase.

Table 5. Expression of IAPs and hTERT according to the grade of dysplasia

Variable	Grade of dysplasia		Pvalue
	Low grade	High grade	
hTERT	41.26 ± 127.45	31.13 ± 94.87	0.968
Survivin	15.48 ± 43.26	4.86 ± 6.15	0.790
XIAP	8.67 ± 31.74	2.86 ± 7.40	0.856
cIAP1	18.73 ± 55.62	41.04 ± 95.96	0.544
cIAP2	1.35 ± 3.81	1.02 ± 2.66	0.585

Values are presented as mean ± SD. IAPs, inhibitor of apoptosis proteins; hTERT, human telomerase reverse transcriptase.

inhibitors, including survivin, cIAP and, XIAP.

The IAP family mainly inhibits caspases 3, 7, and 9, thereby inhibiting apoptosis, whereas cIAP1 and cIAP2 inhibit caspase 8 to suppress apoptosis at the membrane receptor level.¹⁰ In particular, cIAP2 is widely expressed in normal tissues and has a role as a prognostic factor in CRC as well as in evaluating the response to chemotherapy.¹²

While the physiological role of IAPs in normal tissues is unclear, their role in cancerous tissues appears to be different given that they are expressed rapidly and extensively in external tissues with high apoptotic rates.¹³

XIAP might play important roles in tumor progression and could be a potential target for the treatment of human cancers. Therapeutic strategies that interrupt XIAP expression or function have been examined as an adjuvant to conventional chemotherapy and radiation-based cancer therapy.

In addition, survivin is a risk factor for future CRC in colorectal adenoma patients. The biological impact of survivin on CRC risk is consistent with the ability of neoplastic cells to avoid apoptosis and achieve limitless replicative potential.¹⁴

In most tumor cells, telomeres are short and stable, and telomere length is an important indicator of the telomerase activity and is useful for the prognosis of malignant cases, including colorectal neoplasia.¹⁵ hTERT acts as a reverse transcriptase in the elongation of telomeres, which prevents their loss during replication and prevents the end-to-end fusion, recombination, and degradation of chromosomes. In CRC development, hTERT activation occurs during the progression from low- to high-grade IEN in adenomas and steadily increases with progression of dysplasia and colorectal carcinogenesis invasion.¹⁶

In the case of hTERT, one of the main evaluation parameters of this study, mRNA expression is increased by approximately 1.48 times when comparing the surrounding normal mucosa tissue to the expression found in CRC and adenoma tissue.¹⁷

It is not known whether the expression of major IAPs or hTERT is increased in the normal colon tissues of adenoma patients. We analyzed the expression levels of major IAPs, such as XIAP, cIAP1, cIAP2, survivin, and hTERT in both groups by real-time PCR to investigate the factors affecting colon cancer development. In the Mann-Whitney U-test, the cIAP2 levels in the control group and the experimental group were significantly different, but the average ranking was higher in the control group. In this regard, it has been reported that the expression of cIAP2 in RCC tissue is decreased relative to normal tissue by 17.0%.¹⁸ The association of the IAP family and hTERT with carcinogenesis may seem obvious, but we need to study the inverse relationship, as described above.

In conclusion, the expression of major IAPs controlling apoptosis and hTERT, which regulates cell proliferation, on normal colonic mucosa tissues of patients with advanced colorectal adenoma or carcinoma in situ is not different from the control group. The increased expression of these proteins previously reported in cancer patients appears to be initiated at a relatively late stage in the adenoma-carcinoma sequence of colon cancer. There are no differences in the IAP family and hTERT when taking into account the characteristics of advanced adenoma. This study helps to understand the carcinogenic process of colon cancer and identify risk factors for CRC in advanced adenocarcinoma patients.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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