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ORIGINAL RESEARCH

Assessment of Relationship Between Expression of Survivin Protein and Histopathology Diagnosis and Malignancy Severity in Colon Specimen

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Abstract

Background Survivin is a member of the inhibitor of an apoptosis protein family that has been shown to inhibit apoptosis, promote cell proliferation and enhance angiogenesis. In this study, the survivin protein expression in normal, colon polyp, and adenocarcinoma tissues was investigated.

Methods Immunohistochemical staining for nuclear survivin was carried out on 45 normal colon tissue samples, 38 samples of a colonic polyp, and 37 cases of colon adenocarcinoma operated by colonoscopy or colectomy. The percentages of cells that expressed survivin were classified qualitatively into four categories (0, 1+, 2+, and 3+) based on the intensity of staining and the percentage of cells. An area of samples with colon polyp diagnosis or colon adenocarcinoma that had no microscopic pathology was considered as normal tissues.

Results Survivin protein expression was negative in all cases of normal colon tissue samples while it was expressed in 31 out of 38 colon polyp specimens (81.5%) and in 35 out of 37 (94.5%) colon adenocarcinoma samples. Amount of expression in the colon adenocarcinoma ($p < 0.001$) was significantly higher than the amount of expression in the colon polyp. There was not a significant correlation between the survivin protein expression and the low and high grade adenocarcinoma ($p = 0.874$).

Conclusions Survivin protein was not expressed in normal colon tissues and its amount was higher in the colonic adenocarcinoma compared to the colon polyp. Due to the variations in the intensity of expression in colon polyp (changing from negative to + 3), this marker cannot be used for differentiating the polyp from the adenocarcinoma.

Keywords Survivin · Immunohistochemistry · Histopathology · Colon · Tumor · Adenocarcinoma

Introduction

The colon carcinoma is the third most common cancer among men and women, and is also the second leading cause of cancer death. Men and women are affected equally, with an average age of 62 years. In a microscopic examination, adenocarcinoma of the colon shows a wide range of views, which are highly differentiated from neoplasia to various anaplastic tumors [1]. The growth and progression of the tumor in the large intestine depend on the balance between the two factors of cell proliferation and cell death or apoptosis [2] (Fig. 1).

Apoptosis is a cell death pathway in which lethal cells (tumor cells or DNA-damaged cells) activate enzymes that destroy DNA and its nuclear and cytoplasmic proteins and cell membranes. Phagocytic cells remove apoptotic cells before leakage of cell contents [3].

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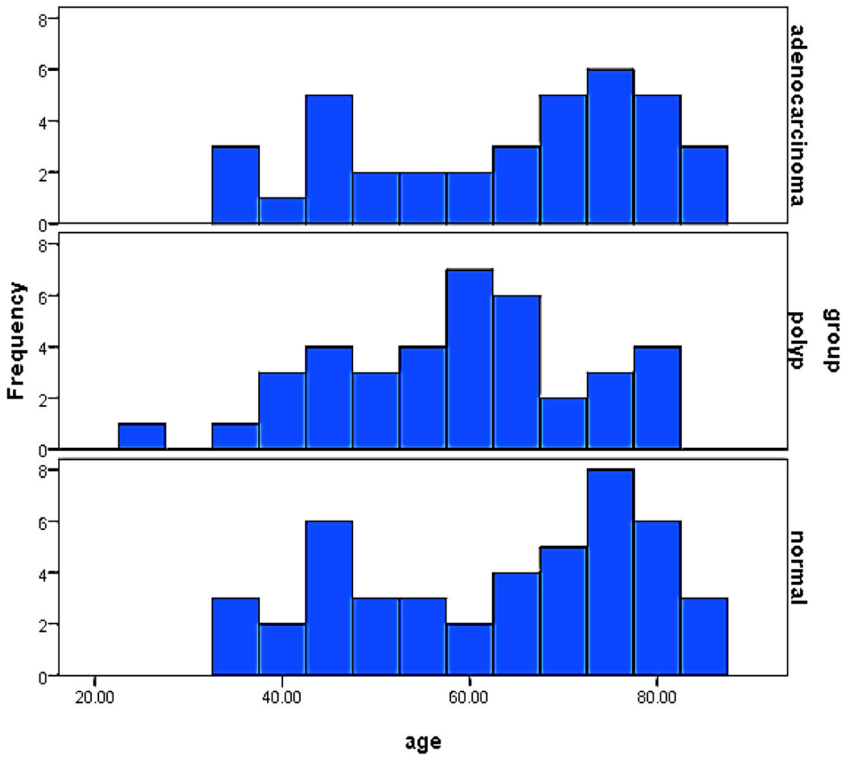

Fig. 1 Frequency of age distribution of patients in the three groups

Apoptosis is initiated and regulated by a cascade of molecular events. Two protein families are involved in the regulation of apoptosis mechanisms: pro-apoptotic protein family and anti-apoptotic protein family or inhibitors of apoptosis proteins (IAP). Since planned death of cells can be considered as one of the defense mechanisms of the human body against cancer, the expression of unregulated anti-apoptotic proteins can lead to elongation of cells; thus, distort mutations and ultimately cause the occurrence and development of cancer [4].

Survivin is an inhibitor of apoptosis that exerts its role in cell proliferation by inhibiting cell-mediated cell death or enhancing mitosis [4, 5]. The survivin gene is located at position 25 on the long arm of chromosome 17, which results in protein coding of 142 amino acids. In contrast to other apoptosis-inhibiting proteins that have a ring-like portion at the end of their carboxylate, survivin has a helical portion of the α-helix similar to that found in microtubule-associated proteins, and with this section, the end of C on speaks to tubulin. Thus, the microtubules of the channel are split and prevent apoptotic cascades [6, 7].

Survivin is the smallest member of the family of IAP [8]. This protein restricts apoptosis by controlling caspase and has the highest expression in the G2/M phase of cell division, which is essential for the progression of mitosis [8, 9].

During the mitosis process, survivin is a component of the chromosomal transfer protein of chromosomal passive proteins (CPPs), which remains complex and essential for biorientation and cell division [10]. Survivin is expressed in the nucleus and cytoplasm of tumor cells [11].

Nuclear survivin appears to promote cell proliferation and the cytoplasmic survivin inhibits apoptosis [12]. Recent evidence suggests that the placement of survivin in the cell (nuclear or cytoplasmic) may be associated with prognosis [13–15].

In this study, by examining the immunomodulatory layer, the paraffin blocks of the survivin colon samples in the colon tissue were examined, and the colon; colon and adenocarcinoma polypeptides were evaluated on the basis of histopathology of the colon. Samples with adenocarcinoma diagnosis of protein expression are compared with malignancy after graduating from American Pathologist College [16]. The relationship between age and sex of patients with survivin protein incidence is also investigated.

Materials and Methods

This case-control study was performed on the samples sent to the Department of Pathology at Shahid Beheshti Hospital in 2014–2015. Adenocarcinoma samples were counted and normal samples were randomly selected and examined. The underlying data of the samples were extracted from the database and patient records. Colon tissue specimens that had undergone a colonoscopy or colectomy were sent to the Department

of Pathology at Shahid Beheshti Hospital. Paraffin blocks and tissue sections with a thickness of 5 μm were prepared according to standard protocols. A number of slides were stained in a similar manner to the hematoxylin-eosin method and the same slides were immunohistochemically stained according to standard formulations. Briefly, the fabric layer was held at 60 °C for 2 h. For rehydration, the layers were then added sequentially to ethanol and distilled water. To eliminate the activity of endogenous peroxidases, the slides were placed in a freshly prepared 3% hydrogen peroxide solution for 20 min. Then, it was washed twice and every 5 min with phosphate buffer saline buffer.

The antigen-retracted slides were then placed in a vessel containing pH = 6 citrate buffer for 10 min in an autoclave at 134 °C and a pressure of 1.5–2 bar. Then, let the screws cool (about 20 min) then wash with distilled water [17–19].

In the next step, the anti-survivin antibody was prepared and added for 1 h. Then, the PBS buffer was washed three times and every 5 min. Then, we added the secondary antibody to the sections for about half an hour at room temperature. At this stage, washing was done with PBS buffer [20–22].

At this stage, the sections were placed in a DAB dilution solution for 10 min (DAB ratio = 1.9, with DAB saturation buffer) and then washed with water. The background was then stained with hematoxylin for 1 min and then washed. For dehydration of slides, absolute alcohol and xylol solutions were used. Finally, the limbs were mounted and ready for examination by optical microscopy [23]. Histopathological diagnosis was determined based on hematoxylin-eosin stained slides and parts of the sample without pathology were considered as normal samples [24–26].

The stained slides were immunohistochemically examined and classified into positive and negative categories, and positive samples were classified according to the color intensity from + 1 to + 3 [27] (Fig. 2):

- Negative or weakly stained samples in less than 10% of the cells: negative
- Poor to moderate staining in 10–29% of the cells: a positive (+ 1)
- Medium to strong dye in 30–49% of the cells: two positive (+ 2)
- Extreme color fastness is about 50% of the cells: three positive (+ 3)

According to the instructions, the cervical squamous cell carcinoma antibody kit was used as a positive control and the normal cervical tissue was used as a negative control. All staining stages of hematoxylin-eosin and

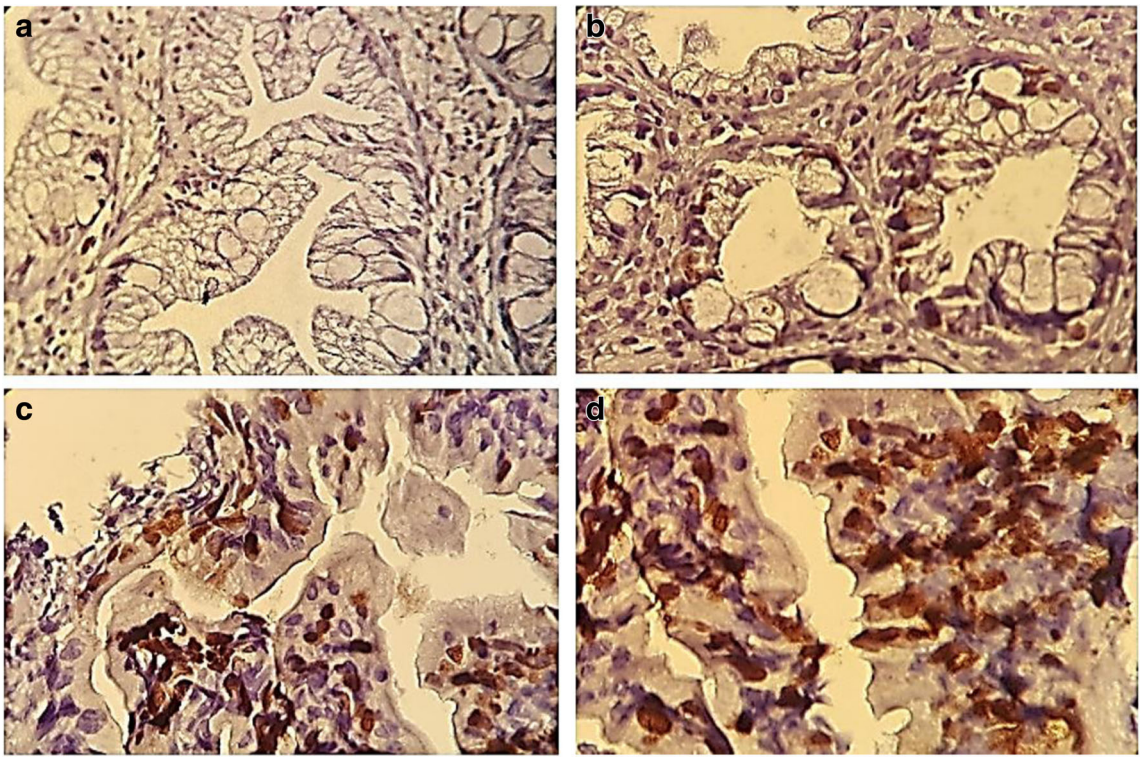

Fig. 2 Negative, less than 10% of the epithelial cells were poorly colored (**a**); positive, 29–10% poor to moderate coloration in epithelial cells (**b**); positive, 49–30% moderate to severe coloration in epithelial cells (**c**); positive, more than 50% of the epithelial cells were stained strongly (**d**)

Table 1 Frequency of sample distribution by gender

Sex	Group						Chi-square test
	Adenocarcinoma		Polyp		Normal		
	Count	Column N %	Count	Column N %	Count	Column N %	
Male	19	51.4%	20	52.6%	25	55.6%	0.925
Female	18	48.6%	18	47.4%	20	44.4%	
Total	37	100.0%	38	100.0%	45	100.0%	

immunohistochemistry were simultaneous and similar to those of the samples tested with positive control and negative control.

The obtained data were recorded for each sample including histopathology, tumor grade, positive or negative immunohistochemistry, and color intensity, and then analyzed using statistical information on the age of the patient.

Statistical Analysis

After collecting the required data, the results were analyzed with the software SPSS 24. The Kolmogorov-Smirnov test was used to evaluate the normality of the data. In this study, parametric tests such as mean analysis variance analysis, chi-square test [28], and correlation tables were used to investigate the independence of multilevel variables and compare survivin protein positivity in the groups studied. Kruskal's nonparametric test is also consistent with nonparametric ANOVA analysis in cases where data is abnormal. In this study, the *P* value (the value of the test error value) is 0.05 with a confidence level of 95%.

Results

The study was performed on 120 colon tissue samples. There were 45 cases of normal colon tissue, 38 cases of polyps, and 37 cases of colon adenocarcinoma (Table 1).

As can be seen from the above table, there is no significant difference in the sex ratio between the three groups.

The mean age of patients in the normal group was 63 years, the 58-year-old polyp group, and the colon adenocarcinoma group 63 years (Table 2).

With regard to the standardization of data for all three groups, we used ANOVA for comparison. The probability of the above table is 0.298 and is greater than 0.05. Therefore, the hypothesis of the mean-graded elderly was included in the colon, polyps, and triple colon adenocarcinoma of the normal colon (Table 3).

Regarding the nonnormalization of survivin data in all three groups, Kruskal-Wallis test is used to determine the nonparametric equation of variance analysis and with the lower probability of a significant probability of 0.05 in the table above, the assumption of the equality of the meanings in the three groups is rejected. Survivin levels were significantly different in adenocarcinoma, polyps, and healthy patients. The mean that survivin protein expression in adenocarcinoma is 2.05, polyp 1.37, and normal tissue was zero (Table 4).

Survivin protein expression expressed in terms of the intensity of coloration of the epithelial cells nucleus was as follows:

Of the 45 normal colon tissues, the dye levels were less than 10% of the epithelial cells in all cases, and 100% of the cases were reported negative.

In 38 samples of colon polyp, 7 cases (18.4%) were negative, 14 cases (36.8%) were positive 1, 13 cases (34.2%) were positive 2, and 4 cases (10.5%) were positive 3. Out of 37 cases of adenocarcinoma of the colon, 2 cases (5.4%) were

Table 2 Frequency of distribution of samples in three groups based on the age of patients and distribution of survivin level

ANOVA test		Groups			*P* value
		Adenocarcinoma colon	Polyp	Normal colon	
Age	Average	63.24	58.45	62.84	0.298
	Standard deviation	15.64	13.69	15.41	

Table 3 Serivine level distribution in the three study groups

Kruskal-Wallis test		Groups			*P* value
		Adenocarcinoma colon	Polyp	Normal colon	
Survivin	Average	2.05	1.37	0.00	< 0.001
	Standard deviation	0.91	0.91	0.00	

Table 4 The frequency of survivin grading distribution in the three study groups

Survivin	Group						Chi-square test
	Adenocarcinoma		Polyp		Normal		
	Count	Column N %	Count	Column N %	Count	Column N %	
.00	2	5.4%	7	18.4%	45	100.0%	< 0.001
1.00	8	21.6%	14	36.8%	0	.0%	
2.00	13	35.1%	13	34.2%	0	.0%	
3.00	14	37.8%	4	10.5%	0	.0%	
Total	37	100.0%	38	100.0%	45	100.0%	

negative, 8 cases (21.6%) were positive 1, 13 cases (35.1%), positive 2, and 14 cases (37.8%) were positive 3 (Table 4).

To examine the impact of the level of survivin levels on groups, we use a cross-sectional table assuming the independence of the two survivin and grouping variables. At a probability value of 0.05, the assumption is zero. Survivin grading showed a significant difference in adenocarcinoma, polyp, and normal groups ($p < 0.001$).

The result of our research indicated that survivin protein level did not show significant differences in adenocarcinoma severity ($p = 0.874$) (Table 5).

Based on the above table, the survivin level is significant in both adenocarcinoma and normal groups ($p < 0.001$) (Table 6).

Based on the above table, it can be concluded that the level of survivin is significant in both polyp and normal groups ($p < 0.001$) (Table 7).

According to the above table, the survivin protein profile in adenocarcinoma and polyp groups also showed that this difference is quite significant ($p = 0.018$) (Table 8).

Discussion

Like other tumors, the growth and development of tumors in the colon depends on the balance between cell proliferation and planned cell death or apoptosis. Uncontrolled expression of anti-apoptotic proteins can result in prolonged cell life, leading to mutational changes and ultimately to the onset and progression of cancer [2]. Survivin is an inhibitor of apoptosis that plays a role in cell proliferation by controlling cell-based cell death or mitosis.

Survivin protein expression in colon tissue was immunohistochemically studied in this study. Colonial adenocarcinomas, colon polyps, and normal colon tissue were compared for survivin protein expression, and in adenocarcinoma colon samples, survivin protein expression was ranked by the College of American Pathologist. The relationship between age and sex of patients with survivin protein was also investigated.

In this study, normal fetuses were used to examine normal tissue along with polyps and adenocarcinoma samples. As with Shariat and colleagues, normal tissue was used in conjunction with adenocarcinoma samples [29]. In some studies, autopsies have been used to obtain normal tissue [30], but our access to autopsy specimens was not possible.

The results of this study were consistent with the standard expression of survivin protein in normal colon tissue similar to previous studies in which normal colon samples were obtained by autopsy [9, 27]. This study also showed that survivin protein is not expressed in the nucleus of normal gland epithelial cells. According to the results, nuclear survivin protein was not expressed in normal colon tissue. In polyps and

Table 5 Comparison of serovinin protein expression in different colon adenocarcinoma severities

Survivin	Cancer grade				*P* value
	low		High		
	Count	Column N %	Count	Column N %	
.00	1	4.2%	1	7.7	0.874
1.00	6	25.0%	2	15.4	
2.00	9	37.5%	4	30.8	
3.00	8	33.3%	6	46.2	
Total	24	100.0%	13	100.0	

Table 6 Comparison of survivin grading in adenocarcinoma and healthy samples

Survivin	Group				*P* value
	Adenocarcinoma		Normal		
	Count	Column N %	Count	Column N %	
.00	2	5.4%	45	100.0	< 0.001
1.00	8	21.6%	0	.0	
2.00	13	35.1%	0	.0	
3.00	14	37.8%	0	.0	
Total	37	100.0%	45	100.0	

Table 7 Comparison of survivin grading in two groups of polyps and normal samples

Survivin	Group				*P* value
	Polyp		Normal		
	Count	Column N %	Count	Column N %	
.00	7	18.4%	45	100.0	< 0.001
1.00	14	36.8%	0	.0	
2.00	13	34.2%	0	.0	
3.00	4	10.5%	0	.0	
Total	38	100.0%	45	100.0	

adenocarcinomas, it was expressed that the expression rate in polyps was 81.6% and adenocarcinoma 94.6%.

In the current study, survivin protein was positively expressed in polyp samples at different rates from negative to 3. In most cases, the level of expression was positive and only in 10.5% of the cases was the expression greater than 50% of the epithelial cells of the glands and was given as 3 positive. In the adenocarcinoma of the colon, the expression varied from negative to 3 positive. Negative specimens consisted of only 4.5%, and most cases reported survivin protein levels at 3%.

In this study, the survivin protein expression in colon adenocarcinoma was higher than the polyp. However, there was no significant relationship with malignancy. In a study by Michiko Shintani et al., there was no significant association between survivin incidence and clinicopathologic parameters [31]. However, in a study by Adamkov et al., out of 113 samples of colon carcinoma, 47 samples expressed survivin protein and the survivin positivity was proportional to gradient elevation [23].

The study found that the likelihood of cancer compared to the polyp increases in survival, so the increase in survival per unit increases the likelihood of cancer by 2.23-fold, and this relationship is quite significant ($p = 0.005$), In this study, the age of patients with survivin protein expression was not significantly correlated.

Table 8 Comparison of survivin grading in adenocarcinoma and polyp samples

Survivin	Group				*P* value
	Polyp		Adenocarcinoma		
	Count	Column N %	Count	Column N %	
.00	7	18.4%	2	5.4	0.018
1.00	14	36.8%	8	21.6	
2.00	13	34.2%	13	35.1	
3.00	4	10.5%	14	37.8	
Total	38	100.0%	37	100.0	

Due to the limited sample size, we did not have a significant relationship between the severities of adenocarcinoma. Also, due to the nature of survivin protein determination, it was not possible to carry out a quantitative investigation between the adenocarcinoma with normal group and normal polyp group.

Based on this study, if the pathology between the polyps and the adenocarcinoma is uncertain, the level of survivin may increase the likelihood of a correct diagnosis. With increasing survivin level, the adenocarcinoma increases.

Recently, survivin gene targeting has been studied in cancer therapy, and in some studies, inhibition of survivin in gene transcription and translation or demyelination of survivin has been used in preclinical or clinical trials [8].

Conclusion

This study examines the expression of survivin protein in adenocarcinomas and colonic polyps, and it appears that expression of this protein in adenocarcinoma colon samples may be used as a biomarker for better patient care in the future. However, extensive studies and clinical trials are required in this context.

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Availability of Data and Materials The dataset used in this study is available with the authors and can be made available upon request.

Authors' Contributions All the authors participated in the study design. TK, AJ, and TM collected and documented the data and assisted in preliminary data analysis. TK, AJ, and TM wrote the initial draft. HHK participated in draft revision, data analysis, and editing of the final draft.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval and Consent to Participate All procedures performed in the study involving human were in accordance with the 1964 Helsinki declaration and ethical standards of the institutional and national research committee of Kashan University of Medical Sciences. The protocol was approved by the research committee of Kashan University of Medical Sciences, Kashan, Iran.

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