ÖZGÜN ARAŞTIRMA / ORIGINAL RESEARCH

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The expression of Beta-Catenin and Sox2 in adenocarcinoma and adenomatous polyps of the colon and their association with clinicopathological parameters

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Abstract

The expression of Beta-Catenin and Sox2 in adenocarcinoma and adenomatous polyps of the colon and their association with clinicopathological parameters

Objective: Our aim was to investigate the immunohistochemical expression of β-catenin and Sox-2 in adenomatous polyps and adenocarcinoma of colon and also to evaluate the effects of these markers in adenoma-carcinoma sequence and their association with clinicopathological parameters.
Method: Fifty-six tubular adenomas with low grade dysplasia (TALGD), 53 tubular adenomas with high grade dysplasia (TAHGD), 44 tubulovillous adenomas (TVA), 29 villous adenomas (VA) and 60 adenocarcinomas were included in the study. The nuclear staining of Sox2 was evaluated as well as both nuclear and cytoplasmic stainings of β-catenin. A semiquantitative scoring was performed. The results were compared between the groups and the relationship of the results with clinicopathological parameters was evaluated.
Results: Nuclear and cytoplasmic β-catenin expressions of the adenocarcinomas were higher than polyps. The expressions in the VA and TVA polyp groups were higher than the expressions in TAHGD and TALGD, respectively. Membranous β-catenin expression in the adenocarcinoma was higher than the polyps except VA. The evaluation between polyp groups with respect to membranous β-catenin staining revealed a statistically significantly difference in favor of VA compared with TVA, TAHGD and TALGD; in favor of TAHGD compared with TVA, in favor of TVA compared with TALGD while it was found statistically significantly higher in TAHGD than TALGD.
Conclusion: The results regarding β-catenin expression of the polyp groups were consistent with the literature. There was a positive correlation between β-catenin expression (nuclear and cytoplasmic) and malignancy. High Sox2 expressions were found correlated with malignancy potential. Large sampling size investigations to be supported by further molecular studies are needed to clarify the effect of Sox2 expression in the sequence of adenoma-carcinoma comprehensively.
Keywords: Adenocarcinoma, Adenomatous polyp, Beta-catenin expression, Colon, Sox2 expression

Öz

Kolon adenokarsinomlarında ve adenomatöz poliplerinde Beta-Catenin ve Sox2 ekspresyonu ve klinikopatolojik parametreler ile ilişkileri

Amaç: β-katenin ve Sox-2'nin kolonun adenomatöz polipleri ve adenokarsinomunda immünohistokimyasal ekspresyonunun araştırılması, adenom-karsinom sekansında bu belirteçlerin yeri ve etkisinin değerlendirilmesidir.
Yöntem: 56 düşük dereceli displazi içeren tübüler adenom(DDDTA), 53 yüksek dereceli displazi içeren tübüler adenom (YDDTA), 44 tübülovillöz adenom (TVA), 29 villöz adenom (VA) ve 60 kolon adenokarsinomu çalışma kapsamına alındı. β-katenin için nükleer ve sitoplazmik, Sox-2 için nükleer boyanma değerlendirildi ve semikantitatif skorlama yapıldı. Bulguların gruplar arasında ve klinikopatolojik parametrelerle ilişkisi değerlendirildi.
Bulgular: Nükleer β-katenin ekspresyonu adenokarsinomda poliplere nazaran anlamlı olarak fazla saptandı. Poliplerde ise VA ile YDDTA arasında VA lehine; TVA ile YDDTA ve TVA ile DDDTA grupları arasında TVA lehine farklıdır. Sitoplazmik β-katenin, adenokarsinom ile VA, TVA, YDDTA ve DDDTA grupları arasında adenokarsinom lehine; VA ile TV, YDDTA ve DDDTA grupları arasında VA lehine; TVA ile YDDTA grupları arasında TVA lehine ve YDDTA ile DDDTA arasında YDDTA lehine istatistiksel olarak anlamlı farklılık bulunmuştur.
Sonuç: Gruplardaki β-katenin sonuçları literatür ile uyumludur. Sox2 ekspresyonu malign potansiyeli destekler niteliktedir. Adenom-karsinom sekansında Sox2'nin etkisinin ayrıntılı açıklanabilmesi için moleküler çalışmalar ile desteklenen geniş serili araştırmalara gereksinim vardır.
Anahtar Kelimeler: Adenokarsinom, Adenomatöz polip, Beta-katenin ekspresyonu, Kolon, Sox-2 ekspresyonu

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INTRODUCTION

Colorectal cancers are the third most common cancer type worldwide. Its incidence increases after 50 years old of age. The risk factors include age, male gender, inflammatory bowel diseases, cigarette smoking and alcohol consumption, dietary habits, obesity and diabetes as well as genetic factors. The most important prognostic factor is the stage of tumor at diagnosis. The treatment options are surgery, neoadjuvant radiotherapy and adjuvant chemotherapy (1, 2).

Adenomas are glandular neoplasms as the known precursors for cancer development that originate from intestinal epithelium and that demonstrate malignancy potential. Its incidence is higher in male gender and increases by advancing age. It has four histological types such as tubular, tubulovillous, villous and flat adenomas (1, 2).

β-catenin is a cytoplasmic protein associated with cadherins and it plays a role in intercellular adhesion. It is an important component of Wnt signaling pathway. APC protein binds to β-catenin and plays a role in breakdown of β-catenin. When APC protein loses its function, cytoplasmic β-catenin accumulates and translocates to nucleus and increases proliferation by activating the transcription of the genes that code MYC and CyclinD1. The mutation of the β-catenin gene has been encountered at various rates in many different cancer types such as hepatoblastoma, intestinal type gastric cancer, endometrial carcinoma, melanoma, anaplastic thyroid carcinoma, prostate carcinoma, Wilms tumor, lung carcinoma and medulloblastoma (3,4,5).

Sox-2 (Sex determining region Y-box2) is the high mobility group box transcription factor belonging to B group of the Sox gene family. Approximately 20 different Sox proteins have been identified. Similarly with others, Sox2 plays a critical role in determining sequence, differentiation and self-renewal capability of the cell. It is found in the cancer stem cells and regulates self-renewal capability of the tumor cells and tumor growth (6). It was encountered to be associated with different cancer types (such as colorectal, breast, ovary, glioblastoma and melanoma). Abnormal Sox2 expression increases cell division in the squamous cell carcinomas of lung and esophagus whereas it inhibits cell division in gastric cancer. In which action mechanism Sox2 expression plays a role in colorectal carcinomas is not clear yet (7).

Our aim was to investigate the immunohistochemical expressions of β-catenin and Sox-2 in adenomatous polyps and adenocarcinoma of colon and also to evaluate the effects of these markers in adenoma-carcinoma sequence and their association with clinicopathological parameters.

METHOD

Fifty-six tubular adenomas with low grade dysplasia (TALGD), 53 tubular adenomas with high grade dysplasia (TAHGD), 44 tubulovillous adenomas (TVA), 29 villous adenomas (VA) and also 60 adenocarcinomas (classical type) were included in the study. Hematoxylin and Eosin (H&E) sections were re-evaluated regarding type and dysplasia grade of the adenomas, differentiation of the carcinomas and pT regardless of the pathology reports by two independent researchers (NE, İG). Patient data were obtained from the pathology reports and operating system of the hospital.

The present study was started after obtaining ethical approval obtained from the Non-Invasive Ethics Committee of Mersin University due to the letter numbered 2015/170 and conducted in accordance with the criteria of Helsinki Declaration were taken into consideration.

Immunohistochemistry

Representative 4-µm sections of 10% formalin-fixed, paraffin-embedded and routinely processed tissue were subjected to study. Immunostaining was performed in all the cases using a monoclonal antibodies directed against β-catenin (Leica Biosystems, Novacastra, clone 17C2, dilution 1:150, Newcastle, United Kingdom) and Sox2 (Abcam, clone 9-9-3, dilution 1:4000, England). Sections were deparaffinized in xylene and dehydrated in graded alcohols. Pretreatment was performed in ethylene diamine tetra-acetic acid at pH 8 and at 98ºC for 20 minutes. This was followed by peroxidase block and 90-minute incubation with the primary antibodies. Diaminobenzidine served as the chromogen.

For nuclear β-catenin; 100 cells were counted in the most densely stained area and number of the stained cells were denominated as %. The density of staining had no influence on scoring. The scores were accepted as 0: negative, <30%: +1 positive, 30-60%:+2 positive and >60%: +3 positive (8). Based on staining density of cytoplasmic β-catenin; the results were graded as 0: no staining, +1 positive: weak, +2 positive: moderate and +3 positive: strong staining. Membranous β-catenin staining was assessed in terms of stained portions of lateral cell membrane from basal to apical direction such as 1/3, 2/3 and 3/3.

For Sox-2; a scores was created by sum of density and percentage of the nuclear staining. Staining density was assessed such that 0: negative, 1: weak, 2: moderate and 3:strong. Staining percentages were graded such as 0:<5%; 1: 5-25%; 2: 26-50%, 3: 51-75% and 4:>75%. The overall score was graded such that 0: 0-1, +: 2-3, ++: 4-5, +++: 6-7 (7).

Statistical Analysis

The statistical analysis of the study data was performed using SPSS 11.5 (for Windows, Chicago, USA) software. The

relationship between staining of nuclear β-catenin and Sox2 and the parameters such as tumor size and age was evaluated by performing One Way Anova Test. The relationship between nuclear β-catenin and the parameters such as gender, localization of the lesions, number of the polyps, tumor differentiation, stage, lymphovascular invasion, Sox2 score, cytoplasmic and membranous β-catenin staining were evaluated by Chi-Square Test. The same method was also used for comparison between Sox2 scores in terms of gender, localization of the lesion, tumor differentiation, stage and number of the polyps. The significance level of the results was determined using Spearman's Rank Correlation Coefficient. The statistical significance level was accepted as p=0.05 in a confidence interval of 95%, therefore the results were accepted statistically significant if p value is less than 0.05.

RESULTS

The case group was composed of 164 male and 78 female patients. The distribution of the patients based on gender and mean age at diagnosis was presented in the Table 1.

Of the adenocarcinomas; 16 were right-sided while 44 were in located the left colon. Tumor size ranged between 2-15cm (mean value: 4.36±1.46). Thirteen, 45 and 2 of the adenocarcinomas were well, moderate and poorly differentiated, respectively. Of the cases; 1, 4, 38 and 17 were in pT1, pT2, pT3 and pT4 stages, respectively. Lymphovascular invasion was encountered in 29 cases (2 well-, 25 moderate- and 2 poorly differentiated). Nineteen and 10 cases were found in pT3 and pT4 stages (Table 2).

The localization, number and histological types (when multiplied) of the adenomatous polyps were presented in the Table 2. There were 2 cases of familial adenomatous polyposis in TALGD group. Of the multiple polyps; 40 and 23 were found to be synchronous and metachronous, respectively.

The results of nuclear, cytoplasmic and membranous staining by β-catenin (Figure 1A-1D) were presented in the Table 3 and the staining results with Sox2 (Figure 2A-B) were shown in the Table 4.

Mean age of the patients with right colon adenocarcinomas was 72.56±11.28 years while mean age of those with left-sided adenocarcinoma was 64.11±14.34 years and there is a statistically significant difference between these values (p=0.038). Moderate-poorly differentiated and advanced stage tumors demonstrated higher rates of lymphovascular invasion (p=0.006, p=0.018, respectively).

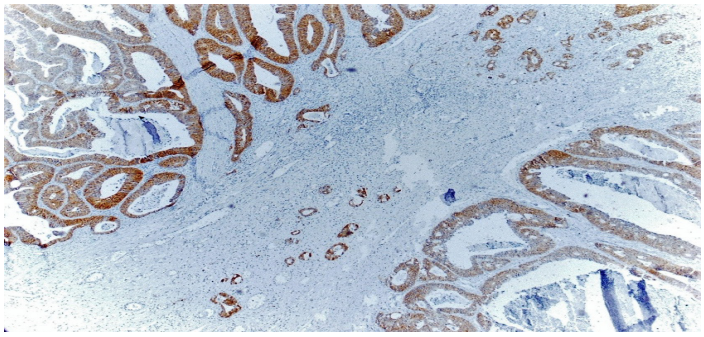

Figure 1a: β-catenin expression in adenocarcinoma (β-catenin, X40)

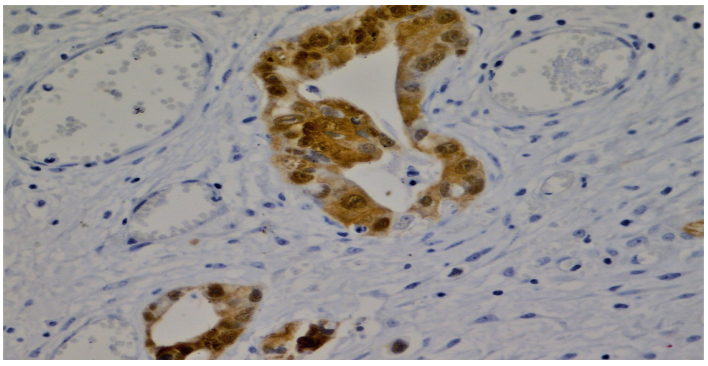

Figure 1b: Nuclear β-catenin staining (β-catenin, X400)

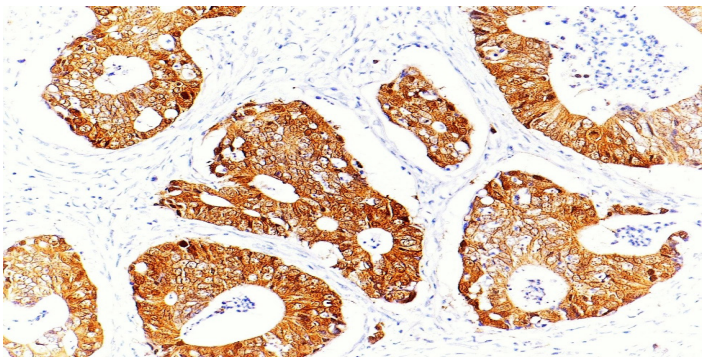

Figure 1c: Cytoplasmic β-catenin staining (β-catenin, X200)

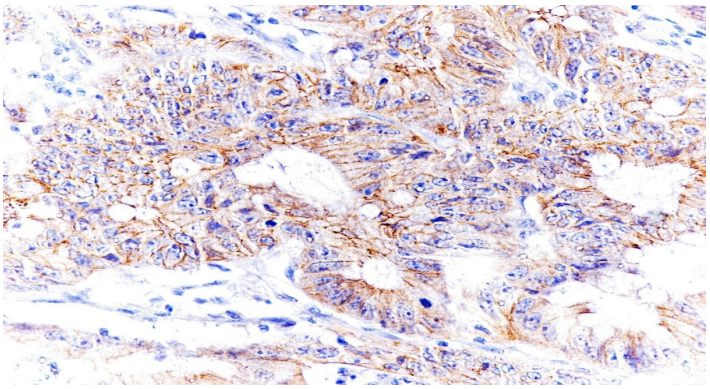

Figure 1d: Membranous β-catenin staining (β-catenin, X200)

Table 1. Demographic characteristics of the cases

	Adenocarcinoma	VA	TVA	TAHGD	TALGD
Number of cases	60	29	44	53	56
Mean age	66.37±14.01	62.59±13.51	65.82±11.26	64.83±13.25	65.63±12.19
Gender					
F	19	12	16	15	16
M	41	17	28	38	40

VA: villous adenoma; TVA: tubulovillous adenoma; TAHGD: tubular adenoma with high grade dysplasia; TALGD: tubular adenoma with low grade dysplasia

For only TVA; all the lesions were localized in the left side of the colon in the females whereas all the lesions were localized in the right side of the colon in the males and there was a significant correlation between gender and localization (p=0.015). In VA, mean age of the cases with right-sided lesions was 49.50±17.97 years while mean age of the cases with left-sided lesions was 64.68±11.81 years and the relationship between localization and age was statistically significant (p=0.034).

Table 2. Histological types, numbers and location of adenomatous polyps

	VA	TVA	TAH-GD	TALGD
Localization				
Right colon	4	6	11	18
Left colon	25	38	42	36
Whole colon	0	0	0	2
Number of polyps				
Single	25	34	27	33
Multiple	4	10	26	21
Histological types	VA, TVA	VA, TVA, TA	VA, TVA, TA	VA, TVA, TA

VA: villous adenoma; TVA: tubulovillous adenoma; TAHGD: tubular adenoma with high grade dysplasia; TALGD: tubular adenoma with low grade dysplasia

Nuclear β-catenin expression was found higher in adenocarcinoma than the polyp groups VA (p=0.014), TVA (p=0.024), TAHGD (p=0.008), TALGD (p=0.004) while nuclear β-catenin expression was detected to be higher in VA and TVA than TAHGD (VA: p=0.035; TVA: p=0,005) and TALGD (p=0.017), respectively.

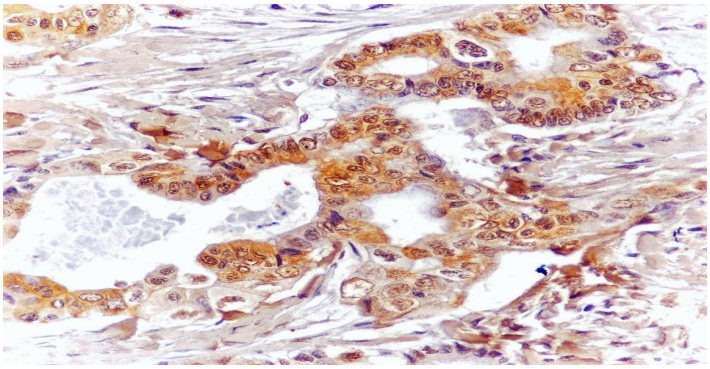

Figure 2a: Sox2 expression in adenocarcinoma (Sox2, X400)

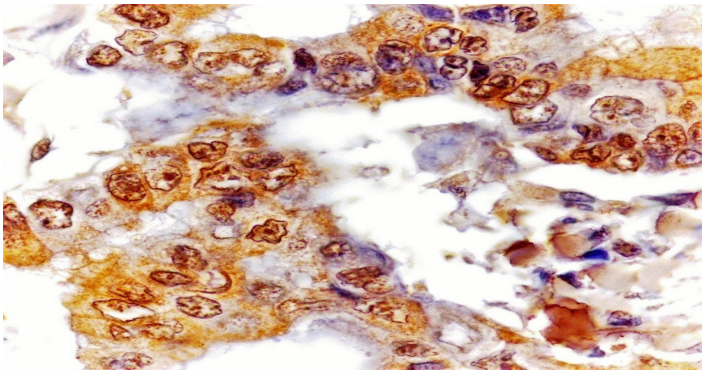

Figure 2b: Nuclear Sox2 staining (Sox2, X1000)

Cytoplasmic β-catenin expression was found significantly higher in adenocarcinoma than VA (p=0.004), TVA (p=0.003), TAHGD (p=0.019) and TALGD (p=0.006) while VA showed a higher cytoplasmic β-catenin expression than TVA (p=0.015), TAHGD (p=0.041) and TALGD (p=0.003) and also a significant difference was found in favor of TVA compared with TAHGD (p=0.009) and in favor of TAHGD compared with TALGD (p=0.017).

Adenocarcinoma demonstrated a statistically significantly higher membranous β-catenin staining than the polyps VA (p=0.013); TVA (p=0.001) and TALGD (p<0. 001) while higher membranous β-catenin staining levels were encountered in favor of VA compared with TVA (p=0.037), TAHGD (p=0.031), TALGD (p=0.004); in favor of TAHGD (p=0.038) than TVA, in

Table 3. The results for nuclear, cytoplasmic and membranous expression of β-catenin between groups

Score	Adenocarcinoma		VA		TVA		TAHGD		TALGD	
Nuclear β-catenin	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
0	29	48.3	22	75.9	31	70.5	49	92.5	50	89.3
1	18	30	5	17.2	10	22.7	1	1.9	5	8.9
2	10	16.7	2	6.9	3	6.8	1	1.9	1	1.8
3	3	5.0	0		0		2	3.8	0	
Cytoplasmic β-catenin										
0	4	6.7	0		8	18.2	7	13.2	14	25.0
1	10	16.7	13	44.8	19	43.2	14	26.4	23	41.1
2	30	50.0	12	41.4	11	25.0	27	50.9	16	28.6
3	16	26.7	13.8	6	6	13.6	5	9.4	3	5.4
Membranous β-catenin										
0	22	36.7	1	3.4	30	68.2	24	46.2	50	89.3
1	2	3.3	4	13.8	2	4.5	1	1.9	0	
2	6	10.0	9	31.0	5	11.4	7	13.5	5	8.9
3	30	50.0	15	51.7	7	15.9	20	38.5	1	1.8

VA: villous adenoma; TVA: tubulovillous adenoma; TAHGD: tubular adenoma with high grade dysplasia; TALGD: tubular adenoma with low grade dysplasia

Table 4. Sox2 nuclear expression in groups

Score	Adenocarcinoma		VA		TVA		TAHGD		TALGD	
Sox2	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
0	34	56.7	24	82.8	24	54.5	38	71.7	41	73.2
1	24	40.0	5	17.2	20	45.5	15	28.3	15	26.8
2	2	3.3	0		0			0	0	
3	0		0		0			0	0	

VA: villous adenoma; TVA: tubulovillous adenoma; TAHGD: tubular adenoma with high grade dysplasia; TALGD: tubular adenoma with low grade dysplasia

favor of TVA compared with TALGD (p=0.009) while TAHGD showed a statistically significantly higher membranous β-catenin staining level than TALGD (p<0.001).

No correlation was determined with respect to nuclear and cytoplasmic β-catenin stainings when an intra-group evaluation was performed in each group. A positive correlation was found between cytoplasmic and membranous β-catenin expressions with respect to adenocarcinoma, TVA and TAHGD (p=0.002, p<0.001 and p=0.027, respectively); membranous staining also increased as cytoplasmic staining intensity.

There was a positive correlation between loss of tumor differentiation and increased nuclear β-catenin expression in adenocarcinoma (p=0.013). The cases with lymphovascular invasion had higher nuclear β-catenin expression than those without lymphovascular invasion (p=0.025).

Due to lack of records, lesion size could be evaluated in only adenocarcinoma group. No significant correlation was present between tumor size and β-catenin expression (0: 4.77±2.52cm; +1, 4.65±1.52cm; +2, 4.30±1.33cm; +3, 3.73±0.46cm). In TALGD, the evaluation based on number of the polyps demonstrated a significant difference in favor of single polyps compared with nuclear β-catenin expression (p=0.041). In VA and TVA groups, single polyps demonstrated higher incidence and higher severity of staining even though this result was not statistically significant. No correlation with localization was determined in none of the groups.

In Sox2 expression, a significant difference was determined in favor of adenocarcinoma (p=0.015) and also TVA (p=0.013) compared with VA. In only TVA group, a statistically significant correlation was present between Sox2 staining and number of polyps (p=0.01). Sox2 expression was not found uncorrelated with tumor size, differentiation, stage, lymphovascular invasion, gender, age, localization of lesions as well as nuclear, cytoplasmic and membranous β-catenin stainings.

DISCUSSION

Adenomas are glandular neoplasms as a known precursor for development of cancer. Malignancy potential of TAs is below 5% whereas malignancy potentials of TVA and VA ranged between 20-25% and 35-40%, respectively (9). Our aim was to investigate the transformation potential of the precursor lesions to cancer based on β-catenin and Sox2 expressions accompanied by clinicopathological characteristics.

The mean age of the patients at the time of diagnosis was consistent with the literature. Adenomatous polyps are usually localized in the left colon and encountered in young ages while right-sided localization is more frequent in advanced age (2,10). However, it has been reported that number of the right-sided polyps increased in the recent years (11). In our study, many of the polyps were localized on the left side. Mean age of the patients with right-sided VAs was relatively lower than those with left-sided VAs. In TVA, the number of the left-sided lesions was higher in the female patients. These findings differ from the literature probably associated with relatively lower number of the cases.

The development of adenoma requires an inactivation in both copies of APC gene due to mutation or epigenetic events. APC binds to β-catenin and induces its breakdown. When APC protein loses its function, β-catenin becomes free and translocates to the nucleus, it activates MYC and CyclinD1 transcription genes. This is followed by KRAS mutation that is one of late-term events, accelerates growth and prevents inhibition of apoptosis. Mutation occurs also in tumor suppressor genes. TP53 tumor suppressor gene is mutated in 70-80% of the cases in the late stages of tumorigenesis. This gene is rarely affected in the adenomas (3).

We have determined that adenocarcinoma revealed statistically significantly higher nuclear and cytoplasmic β-catenin expressions than all of the polyp groups. Nuclear β-catenin staining was significantly higher in VA than TAHGD while TVA revealed significantly higher expression than TAHGD and TALGD. It is known that malignancy potential is elevated increasing size, dysplasia grade and villous morphology in the adenomateous polyps. Even though, the highest nuclear β-catenin expression is encountered in the adenocarcinomas, the increase in the level of nuclear and cytoplasmic β-catenin as risk for malignancy increases in the polyps can be explained by the increasing accumulation of β-catenin towards the latest stages within the stepwise progression of adenoma-carcinoma sequence. Herter et al. have obtained similar outcomes with our study. Differences in immunoreactivity between tumor cells may be associated with tumor heterogeneity (12). Iwamoto et al. have also demonstrated β-catenin expression in adenocarcinoma and polyps however they have also suggested that loss of APC function is not absolutely responsible for accumulation of β-catenin and that various mechanisms aside from APC mutation may be also effective for accumulation of β-catenin (13).

Cytoplasmic and membranous β-catenin staining of adenocarcinoma was significantly higher than the polyps. We have encountered a significant difference between the cytoplasmic expression levels of the adenomatous polyps VA, TVA, TAHGD and TALGD from the highest towards to the lowest respectively in the intergroup comparison. Membranous β-catenin staining was also higher in VA group than the other polyp groups. A positive correlation was identified between cytoplasmic and membranous β-catenin stainings in the adenocarcinoma, TVA and TAHGD groups. These results support the relationship of structural characteristics and grade of dysplasia with malignancy potential. β-catenin is the intracellular component of the cadherin proteins that function in cellular adhesion, it binds to α-catenin and establishes the communication between the adhesions complex and cytoskeleton. Herter et al. have shown that adenocarcinoma cells manifest membranous accumulation independently of cytoplasmic and nuclear accumulation of β-catenin. They have also determined that there were numerous tumor cells that did not reveal cytoplasmic and nuclear staining similarly with colonic cells (12).

We have found a poor differentiation and lymphovascular invasion statistically significantly correlated with β-catenin staining. Loss of differentiation may occur in focal areas and develop due to additional mutations. Accumulation of β-catenin increases until additional mutations occur. Mojarad et al. and Neumann et al. have proposed that a new potential prognostic determinant independent of differentiation and stage may be present although they have identified no significant correlation between β-catenin and tumor differentiation (8, 14). Hlubek et al. have shown that β-catenin staining is heterogeneous therefore cytoplasmic and membranous stainings are predominant in the central cells of the tumor whereas nuclear staining is higher in the periphery and they have attributed this result to synthesis of different targeted genes of Wnt/ β-catenin signaling pathway in these two different areas (8,14,15). Some studies have suggested that increased nuclear β-catenin expression has a weaker prognostic value in the literature whereas other studies accept reduced β-catenin expression to be associated

with worse prognosis (16-18).

In our study, increased nuclear β-catenin staining level was significant in TALGD in favor of single polyps. Even though, this result was not statistically significant, the number of the stained cases and severity of staining were higher in VA and TVA. It is known that single polyps have higher malignancy potential. It has been noted that accumulation of β-catenin begins from the nucleus in the early developmental stages of the adenomas (12).

Sox factors regulate the cellular events such as tissue specification, organ development, stem cell homeostasis and development of cancer (19). They may activate or inhibit transcription depending on cellular content or related proteins. As well as their functions independent of Wnt, they have also a regulator role on Wnt in normal development and disease course. Some of those factors suppress the efficacy of β-catenin whereas some Sox factors elevate the transcriptional activity of β-catenin. On the other hand, the expression of some Sox genes is regulated by Wnt and this feature provides a feedback mechanism. They may have different effects on tumors (20). Sox factors have been detected in normal small intestine and bowel. Sox2 is found in the small intestine, colon, esophagus and stomach. It plays a role in normal epithelial proliferation and continuity of epithelial cells in the different localizations of the gastrointestinal system (21-25).

Neumann et al. have found that high levels of Sox2 and β-catenin expressions are associated with metastasis of distant organ and lymph node (9). Invasion and metastasis are caused by the migrating tumor stem cells. β-catenin is one of the factors which activate and progress the features of colorectal cancer stem cells, Sox2 stimulates these cells to gain embryonic stem cell features. Therefore, it has been suggested that β-catenin expression accompanied with Sox2 may cause malignant progression by inducing stem cell features in colorectal tumor cells (8). In vitro studies have shown that Sox2 suppresses transcriptional activity of β-catenin by binding directly to the β-catenin/Tcf complex and that Sox2 transcription is also regulated by β-catenin in a similar pattern. It has been also determined that Sox2 expression reduces growth rate in vivo and in vitro colorectal tumor cell cultures (26). It has been suggested that dominance between these two effects of Sox2 determines the biological behavior of the tumor.

Cytoplasmic staining was mentioned in adenocarcinoma in some of the studies, the evaluations were performed based on nuclear staining. The rationale for the acceptance of nuclear staining as a positive prognostic factor is the fact that Sox2 is a nuclear transcription factor. Molecular methods were used as well as immunohistochemical methods in some of the studies by Sox2 (27,28). Similar data were obtained by these methods (29). We have encountered no study in the literature that clarified the biological importance of cytoplasmic staining. We also observed cytoplasmic staining in some of the tumor cells. No staining was detected in normal mucosa.

We found that Sox2 staining was significant in adenocarcinoma and TVA. There was a correlation between only staining and number of polyps in TVA group. Higher staining was observed in the single TVA polyps. It should be noted that Sox2 scores were low in our all cases. The statistically insignificant results obtained in the other polyps may have resulted from relatively low sampling size and inequality between the groups regarding the number of our cases. No correlation was found between nuclear β-catenin and Sox2 staining. Some studies have suggested similar outcomes (8). Park et al. found high levels of Sox2 expression in mucinous cancer of colon (29). Various outcomes of the studies may lead to consideration that tumors may be heterogeneous detected in the different geographical regions. More detailed studies are needed to clarify the relationship between different staining levels.

Limitations

The small sampling size and inclusion of the cases obtained from a single institution (Mersin University Medical Faculty) were the limitations of our study.

CONCLUSION

Colon cancers are very common among all cancers and they frequently develop from dysplastic adenomas in a long period of time. We have concluded that β-catenin and Sox2 expressions are effective in sequence from adenoma to cancer. Further larger-sized studies conducted on groups including equal numbers of cases to be supported by advanced molecular studies are needed to clarify the pathogenesis more comprehensively.

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Peer-Review

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Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article..

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Previous Publishing

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"immunoexpression and significance of b-catenin and sox2 in adenomatous polyps and adenocarcinomas of colon".

This study was prepared by rearrangement of the specialty thesis by first author, entitled as "kolon poliplerinde adenom/karsinom sekansında sox-2 ve beta-katenin ekspresyonunun yeri ve tanıya katkısının araştırılması" and dated 2016.

Ethical Declaration

Ethical permission was obtained from the Mersin University, Medical Faculty Clinical Research Ethics Committee for this study with date 11.06.2015 and number 170, and Helsinki Declaration rules were followed to conduct this study.

Authorship Contributions

Concept: N.G., İ.G., E.S.L., E.U., Design: N.G., İ.G., E.S.L., T.C.,.Data Collection or Processing: N.G., İ.G., D.G., T.C., Analysis or Interpretation: N.G., İ.G., E.S.L., D.G., E.U., Literature Search: N.G., E.S.L., D.G., E.U., T.C., Writing: N.G., İ.G.

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