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Sirtuin 1 and Sirtuin 2 Gene Expressions in Colorectal Cancers

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ABSTRACT

Objective: This study aimed to evaluate the immunoreactivity of sirtuin 1 (SIRT1) and sirtuin 2 (SIRT2) in colorectal cancer tissues using immunohistochemistry and to investigate their potential roles in the diagnosis and prognosis of colorectal cancer.

Materials and Methods: Colon specimens from 104 patients diagnosed with colorectal adenocarcinoma at SBÜ Kartal Dr. Lütfi Kırdar City Hospital between 2016 and 2021 were analyzed. Immunohistochemical staining was performed to assess the immunoreactivity of SIRT1 and SIRT2. Associations between the clinicopathological parameters of colorectal cancer patients and the expression levels of SIRT1 and SIRT2 (categorized as low or high) were examined.

Results: SIRT1 expression was significantly associated with K-RAS mutations (p=0.021) but showed no significant association with N-RAS (p=0.114) or B-RAF (p=0.624) mutations. SIRT2 expression levels were significantly associated with TNM stage (p=0.043), presence of metastasis (p=0.004), K-RAS mutations (p=0.047), and N-RAS mutations (p=0.020). Co-expression of SIRT1 and SIRT2 was significantly correlated with TNM stage (p=0.041), presence of metastasis (p=0.012), and mutations in K-RAS (p=0.028) and N-RAS (p=0.022).

Conclusion: SIRT2 expression levels were significantly correlated with TNM classification, as well as the presence of metastasis. In contrast, SIRT1 expression was not significantly associated with these parameters. Both SIRT1 and SIRT2 showed a statistically significant relationship with K-RAS mutations, highlighting their potential roles in the molecular pathology of colorectal cancer.

Keywords: Cancer, colon, colorectal, rectum, Sirtuin 1, Sirtuin 2.

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INTRODUCTION

Colorectal cancer (CRC) ranks among the most prevalent malignant tumors globally.[1] Recent research has focused on identifying novel molecular indicators for CRC. Among these, epigenetics has emerged as a promising area, as aberrant epigenetic modifications not only contribute to cancer formation but also play a role in its progression. Histone deacetylases, a class of epigenetic regulators, have been implicated in these processes in various cancers.[2]

Sirtuins function as NAD+-dependent class III histone deacetylases. Among them, Sirtuin 1 (SIRT1) has been the subject of extensive research due to its role in modulating a wide range of biological activities.[3] While some studies suggest SIRT1 functions as a tumor suppressor, others provide evidence for its oncogenic properties. Altered expression of sirtuins has been identified across a range of cancer types.[4–7] In gastrointestinal malignancies, SIRT1 levels are frequently found to be increased;[5] however, its association with survival outcomes remains inconclusive, with studies reporting conflicting results.[6] Similarly, Sirtuin 2 (SIRT2) plays a key role in regulating the cell cycle and repairing DNA damage. Depending on its localization and function, SIRT2 may behave as a tumor suppressor or an oncogene.

Although sirtuin dysregulation has been reported in multiple cancer types, the relationship between sirtuin gene expression and clinical parameters in CRC remains unclear. This study aimed to investigate the expression of sirtuin genes in CRC and analyze their associations with demographic and clinical data, including patient age, gender, TNM classification, tumor histological type, tumor localization, and lymphatic metastasis.

MATERIALS AND METHODS

Patient Selection

This study included colon resection specimens from 104 patients diagnosed with colorectal adenocarcinoma at SBÜ Kartal Dr. Lütfi Kırdar City Hospital between 2016 and 2021. Ethical approval was obtained from the local ethics committee (approval number: 2021/514/200/11), and the study was supported by SBÜ under project code 2022/069. This research complies with the guidelines for human studies and the research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The study protocol was approved by the institute's committee on human research.

Patient data were retrieved using the hospital's automation system. Clinical staging was performed based on the 8th Edition TNM classification.[1]

The evaluated parameters included age at diagnosis, gender, tumor location (right vs. left colon), TNM classification, lymphovascular invasion, perineural invasion, metastasis/recurrence, and immunohistochemical SIRT1 and SIRT2 expression (low vs. high).

Inclusion criteria required a confirmed diagnosis of colorectal adenocarcinoma and sufficient sample availability for SIRT1 and SIRT2 immunohistochemistry. Exclusion criteria included the absence of paraffin-embedded blocks in the pathology archive or the presence of a second primary tumor in the patient.

KEY MESSAGES

- Although sirtuin dysregulation has been reported in multiple cancer types, the relationship between sirtuin gene expression and clinical parameters in colorectal cancer remains unclear.
- There was a significant correlation between SIRT2 expression levels and key prognostic indicators, including TNM classification, as well as the presence of metastases. SIRT1 expression did not show a significant relationship with these parameters.
- Both SIRT1 and SIRT2 were significantly associated with KRAS mutations in CRC.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue specimens and hematoxylin and eosin (H&E)-stained sections were retrieved from the pathology archive. For cases without pre-existing H&E slides, new sections were prepared to identify appropriate blocks containing both tumor and normal tissue. One diagnostic block per case was selected.

The SIRT1 (B-7, mouse monoclonal antibody, sc-74465) and SIRT2 (A-5, mouse monoclonal antibody, sc-28298) antibodies were used for staining. Optimal dilution ratios were determined as 1:300 for SIRT1 and 1:100 for SIRT2 through preliminary trials. Internal and external controls were selected based on staining scores described by the Human Protein Atlas (http://www.proteinatlas.org/).

Immunohistochemical staining was implemented using the Ventana Medical System-Benchmark Ultra/ISH Staining platform and the Ultraview Universal DAB Detection Kit. The staining protocol included: -Sectioning of paraffin blocks into 4 µm-thick slices on positively charged slides. -Incubation at 70°C for 1 hour. -Antigen retrieval using ethylenediaminetetraacetic acid (EDTA) at pH 8 (CC1). -Primary antibody incubation: SIRT1 (1:300 dilution) and SIRT2 (1:100 dilution) for 2 hours. -Background staining with Harris Hematoxylin for 16 minutes. -Application of Bluing Reagent for 4 minutes. -Washing with detergent and water, followed by rinsing in absolute alcohol. -Drying and coverslipping with xylene-based sealant.

After staining, all slides were examined under a light microscope.

Evaluation of Sirtuin Expressions

A semi-quantitative scoring system was utilized to assess SIRT1 and SIRT2 expression, based on previously established methods.[8,9] Staining extent was categorized as follows: 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). Staining

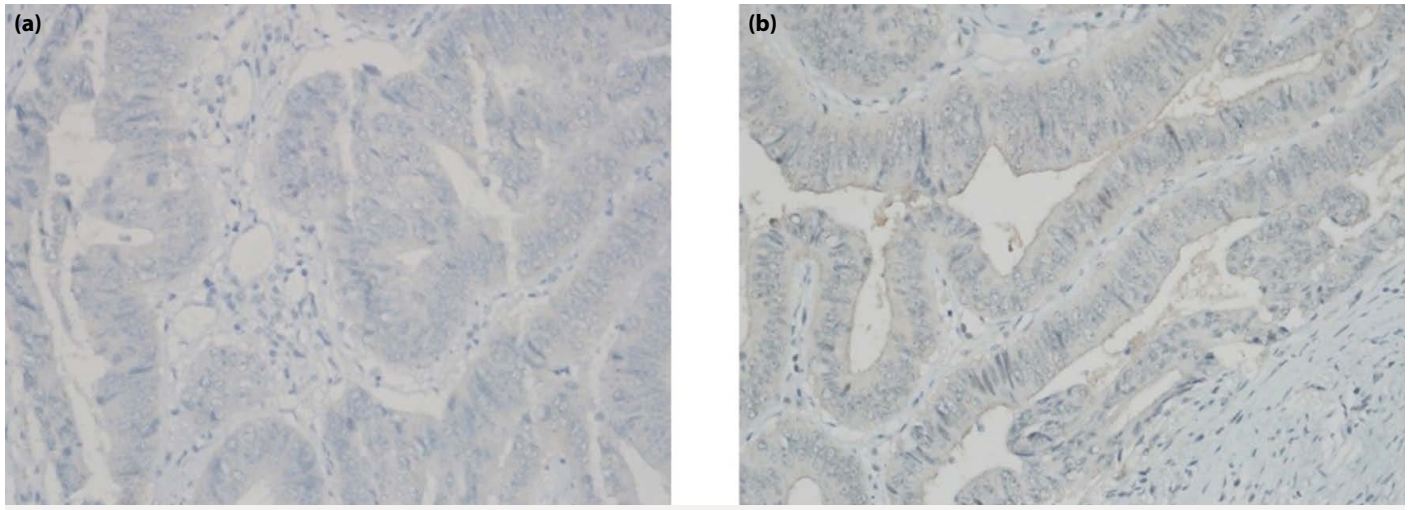

Figure 1. SIRT1 and SIRT2 negative patients with colorectal cancer, **(a)** SIRT1 negative IHC X400, **(b)** SIRT2 negative IHC X400.

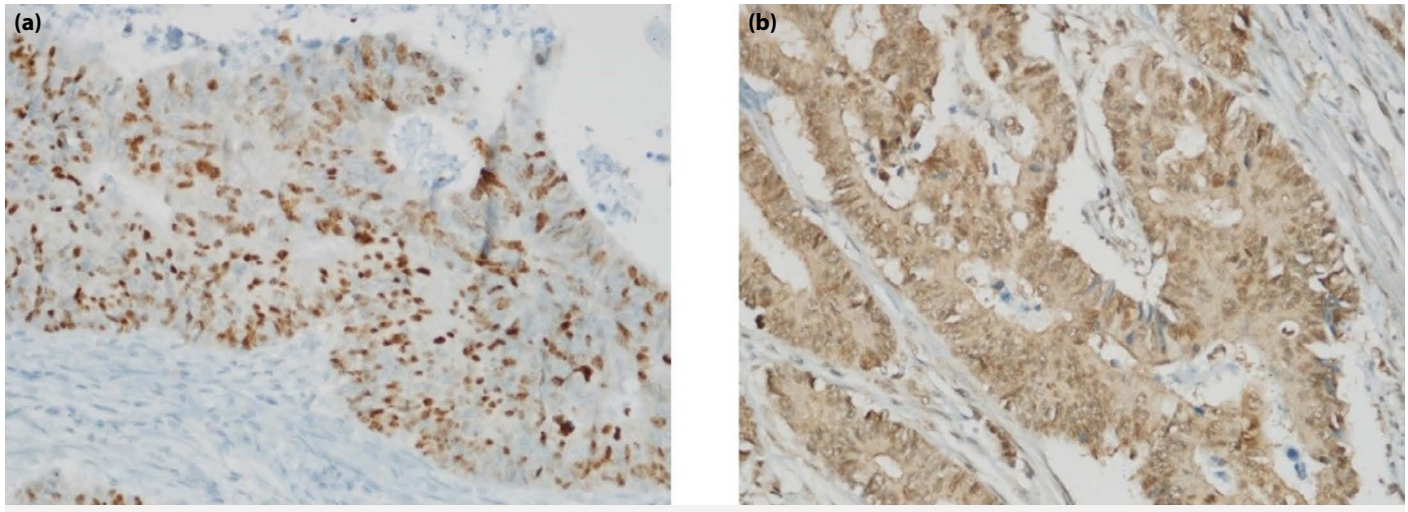

Figure 2. SIRT1 and SIRT2 positive patients with colorectal cancer, **(a)** SIRT1 positive IHC X400, **(b)** SIRT2 positive IHC X400.

intensity was rated as 0 (negative), 1 (weak), 2 (moderate), or 3 (strong). The final immunohistochemistry score (Allred score) was obtained by combining the extent and intensity scores.

SIRT1 and SIRT2 expression levels were classified as:

-High expression: score >3 and low expression: score ≤3

Negative and positive staining patterns for SIRT1 and SIRT2 are shown in Figures 1 and 2.

Statistical Analysis

Demographic, clinical data were analyzed using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics (percentages, means, medians, distributions, and graphs) were calculated for the study group. Comparisons between groups were performed using the Student's t-test for parametric variables and the Mann–Whitney U test for nonparametric variables, while categorical data were analyzed with the Chi-square test. A p-value <0.05 was considered statistically significant.

RESULTS

Demographic features of 104 CRC patients have been given in Table 1. The mean age was 61.95±11.27 years, with patients ranging from 24 to 83 years old. The majority of patients were male (67.3%), and most tumors were localized in the left colon (78.8%). Histological evaluation showed that 76.9% of tumors were moderately differentiated, with smaller proportions of

well-differentiated (3.8%) and poorly differentiated tumors (19.2%). Tumor extension (T stage) indicated that 57.7% of cases were T3, while 37.5% were T4. Lymphovascular invasion was present in 70.2% of cases, and perineural invasion was identified in 61.5%. Regarding lymph node involvement, 38.5% of patients were N2, and 34.6% had no lymph node involvement (N0). Distant metastases were found in 17.3% of patients. Based on TNM staging, most patients were in stage 3 (51.9%) or stage 2 (27.9. Molecular analysis revealed that 46.2% of patients had K-RAS mutations, 7.7% had N-RAS mutations, and 1.9% had B-RAF mutations.

Examination of Clinicopathological Parameters According to SIRT1 and SIRT2 Scores

The analysis of SIRT1 expression levels (low vs. high) is presented in Table 2. Age, gender distribution, tumor localization, histological grade, TNM staging, metastasis, lymph node involvement, tumor extension, lymphovascular invasion, and perineural invasion showed no significant associations with SIRT1 expression ($p>0.05$). However, a higher proportion of poorly differentiated tumors was observed in the high SIRT1 expression group, and a trend toward higher TNM stages was noted in the low-expression group ($p=0.057$). Molecular analysis revealed a significant association between SIRT1 expression and K-RAS mutation status ($p=0.021$), with K-RAS mutations more prevalent in the low-expression group. No significant relationships were found with N-RAS ($p=0.114$) or B-RAF ($p=0.624$) mutations.

Table 3 details the relationship between SIRT2 expression levels (low vs. high). Patients with high SIRT2 expression were more likely to be male ($p=0.03$). Significant differences were found in TNM staging, where high SIRT2 expression was associated with earlier TNM stages ($p=0.043$). Notably, metastasis was significantly less frequent in the high SIRT2 expression group ($p=0.004$). Molecular analyses showed that high SIRT2 expression was more common in K-RAS wild-type tumors ($p=0.047$) and N-RAS mutant tumors ($p=0.020$), while no significant relationship was found with B-RAF mutations ($p=0.802$). Other clinicopathological parameters, such as age, tumor localization, histological grade, lymph node involvement, tumor extension, lymphovascular invasion, and perineural invasion, did not show significant differences between SIRT2 expression groups.

Determining the Relationship of SIRT1 and SIRT2 Concordance with Clinical Parameters

Table 4 examines clinicopathological parameters based on the concordance of SIRT1 and SIRT2 expression, categorized into four groups: both low expression (-/-), both high expression (+/+), SIRT1 high and SIRT2 low (+/-), and SIRT1 low and SIRT2 high (-/+).

Age distribution did not vary considerably between the groups ($p=0.471$). Gender distribution showed a near-significant trend ($p=0.076$), with more males in the (+/+) and (+/-) groups. Tumor localization and histological grade showed no significant associations with concordance groups ($p=0.237$ and $p=0.41$), with most tumors located in the left colon and moderately differentiated across all groups.

Significant differences were observed in TNM staging ($p=0.041$). Advanced TNM stages (3 and 4) were more frequent in the (+/+) group, whereas earlier stages (1 and 2) were more common in the (+/-) and (-/+) groups.

Consistently, metastasis was significantly less common in the (+/+) group ($p=0.012$), while it was more frequent in the (-/+) and (-/-) groups. This indicates that although (+/+) patients tended to present with locally advanced disease, they did not show a proportionally high frequency of distant metastasis. Lymph node stage, microscopic tumor extension, lymphovascular invasion, and perineural invasion did not reveal any notable differences between concordance groups ($p>0.05$).

Molecular analysis revealed significant associations with K-RAS and N-RAS mutations. The (+/+) group had a higher prevalence of wild-type K-RAS ($p=0.028$) and N-RAS ($p=0.022$), whereas the (+/-) and (-/+) groups exhibited more mutations in both genes. No significant association was observed with B-RAF mutations ($p=0.519$).

DISCUSSION

Colorectal cancer remains among the top three causes of cancer-related deaths worldwide.[10] In the current research, we have reported the expression of SIRT1 and SIRT2 genes in 104 patients with colorectal adenocarcinoma and analyzed their relationship with various clinical parameters.

In our study, 38.5% of colorectal cancer patients showed low SIRT1 expression, while 61.5% had high expression levels. In a study by Hong et al.,[11] which included 265 patients, an elevation in SIRT1 expression was observed in 24.5% of cases. While SIRT1 is the most researched of all sirtuins, literature reports conflicting results, with both increased and decreased expression found in colorectal cancers.[12,13] Recent research suggests that the dual roles of SIRT1 could be linked to its intracellular localization. Initially identified as a nuclear protein, SIRT1 has been shown to possess nuclear export signals, facilitating nucleocytoplasmic migration.[14] Additionally, evidence suggests that its different subcellular localizations may influence substrate specificity in both normal and cancer cells.[15]

Table 1. Demographic and clinical characteristics of the study group

	Frequency	Percentage
Gender		
Female	34	32.7
Male	70	67.3
Localization of tumor		
Right	22	21.2
Left	82	78.8
Histological grade		
Well	4	3.8
Moderate	80	76.9
Poor	20	19.2
Microscopic tumor extension/T stage		
2	5	4.8
3	60	57.7
4	39	37.5
Lymphovascular invasion		
No	31	29.8
Yes	73	70.2
Perineural invasion		
No	40	38.5
Yes	64	61.5
Lymph node stage		
0	36	34.6
1	28	26.9
2	40	38.5
Metastasis		
No	86	82.7
Yes	18	17.3
TNM		
1	2	1.9
2	29	27.9
3	54	51.9
4	19	18.3
K-RAS		
Wild	56	53.8
Mutant	48	46.2
N-RAS		
Wild	96	92.3
Mutant	8	7.7
B-RAF		
Wild	102	98.1
Mutant	2	1.9

TNM: Tumor, node, metastasis staging system; K-RAS: Kirsten rat sarcoma viral oncogene homolog; N-RAS: Neuroblastoma RAS viral (v-ras) oncogene homolog; B-RAF: B-Raf proto-oncogene, serine/threonine kinase.

Table 2. Comparison of SIRT1 expression levels and clinical parameters

	SIRT1 expression levels		p
	Low (n=40)	High (n=64)	
Age, (mean)	62.75±11.74	61.45±11.04	0.571
Gender			0.574
Female	13	21	
Male	27	43	
Localization of tumor			0.068
Right	12	10	
Left	28	54	
Histological grade			0.145
Well	3	1	
Moderate	32	48	
Poor	5	15	
TNM			0.057
1	2	0	
2	10	19	
3	17	37	
4	11	8	
Metastasis			0.116
No	30	56	
Yes	10	8	
Lymph node stage			0.611
0	15	21	
1	12	16	
2	13	27	
Microscopic tumor extension/T stage			0.588
2	3	2	
3	22	38	
4	15	24	
Lymphovascular invasion			0.664
No	13	18	
Yes	27	46	
Perineural invasion			0.539
No	17	23	
Yes	23	41	
KRAS			0.021
Wild	16	40	
Mutant	24	24	
NRAS			0.114
Wild	39	57	
Mutant	1	7	
BRAF			0.624
Wild	39	63	
Mutant	1	1	

SIRT1: Sirtuin 1; TNM: Tumor, node, metastasis staging system; K-RAS: Kirsten rat sarcoma viral oncogene homolog; N-RAS: Neuroblastoma RAS viral (v-ras) oncogene homolog; B-RAF: B-Raf proto-oncogene, serine/threonine kinase. Comparisons between 'age' groups were performed using the Mann–Whitney U test, while categorical data were analyzed with the Chi-Square or Fisher's Exact test, as appropriate.

Table 3. Comparison of SIRT2 expression levels and clinical parameters

	SIRT2 expression levels		p
	Low (n=43)	High (n=61)	
Age, (mean)	59.74±10.49	63.51±11.63	0.094
Gender			0.03
Female	19	15	
Male	24	46	
Localization of tumor			0.635
Right	8	14	
Left	35	47	
Histological grade			0.779
Well	2	2	
Moderate	34	46	
Poor	7	13	
TNM			0.043
1	0	2	
2	11	18	
3	19	35	
4	13	6	
Metastasis			0.004
No	30	56	
Yes	13	5	
Lymph node stage			0.465
0	15	21	
1	14	14	
2	14	26	
Microscopic tumor extension/T stage			0.889
2	2	3	
3	26	34	
4	15	24	
Lymphovascular invasion			0.097
No	9	22	
Yes	34	39	
Perineural invasion			0.505
No	17	23	
Yes	26	38	
KRAS			0.047
Wild	18	38	
Mutant	25	23	
NRAS			0.020
Wild	43	53	
Mutant	0	8	
BRAF			0.802
Wild	42	60	
Mutant	1	1	

SIRT2: Sirtuin 2; TNM: Tumor, node, metastasis staging system; K-RAS: Kirsten rat sarcoma viral oncogene homolog; N-RAS: Neuroblastoma RAS viral (v-ras) oncogene homolog; B-RAF: B-Raf proto-oncogene, serine/threonine kinase. Comparisons between 'age' groups were performed using the Mann–Whitney U test, while categorical data were analyzed with the Chi-square or Fisher's Exact test, as appropriate.

SIRT1 expression levels showed no significant correlation with basic clinical parameters in our study; however, a notable association with K-RAS mutations was observed. Similarly, Hong et al.[11] reported a relationship between SIRT1 and vascular invasion, though no meaningful correlation was observed with clinicopathological parameters or survival outcomes. Contrarily, other studies have reported associations between SIRT1 and clinical parameters. For instance, Lv et al.[7] observed elevated SIRT1 expression in advanced tumors, lymph node involvement, and metastatic cases, along with a significant link to poorer survival and shorter disease-free interval.

SIRT2 expression was low in 41.3% and high in 58.7% of patients, with higher levels observed in males. There was a correlation between SIRT2 expression and TNM stage and metastasis. While the sirtuin family is associated with metastasis in colorectal cancer (CRC), the specific direction and extent of this relationship remain debated.[16] Zhang et al.[17] noted that SIRT2 is down-regulated in CRC biopsy specimens compared to adjacent normal tissues, with decreased SIRT2 expression linked to poor clinicopathological features and prognosis. Predominantly localized in the cytoplasm of colonic epithelial cells, SIRT2 downregulation has been associated with adverse outcomes.[17] Cheon et al.[18] showed that AK-1, a SIRT2-specific inhibitor, slowed cancer progression by halting the cell cycle in colon carcinoma cells. Additionally, Hu et al.[19] found that SIRT2 inhibition restricts tumor angiogenesis. Collectively, these findings suggest that SIRT2 may have dual and potentially opposing roles in CRC progression. A significant limitation in the existing literature is the small sample size of studies and the predominance of mRNA-level analyses from online databases, rather than protein-level investigations.[20]

When the coexistence of SIRT1 and SIRT2 expression levels was analyzed in relation to clinical parameters, a significant association was observed with TNM stage and the presence of metastasis.

A noteworthy finding of our study was the notable association between K-RAS mutations and the expression of both SIRT1 and SIRT2, including their co-expression groups. The KRAS oncogene is a central focus in cancer research since its discovery.[21] KRAS mutations are among the most critical markers for determining resistance to epidermal growth factor receptor (EGFR) inhibitors.[22] Beyond being an unfavorable prognostic marker, KRAS mutations have emerged as a promising area of exploration for therapeutic strategies.[22,23] Currently, treatment alternatives for patients with advanced KRAS mutant microsatellite-stable colorectal cancer include chemotherapy schemes like FOLFIRI, FOLFOX, or FOLFOXIRI, often paired with anti-vascular endothelial growth factor drugs.[24]

Table 4. Comparison of SIRT1 and SIRT2 concordance with clinical parameters

	SIRT1 / SIRT2 concordance				p
	+/ + (n=39)	+/ - (n=25)	-/ + (n=22)	-/ - (n=18)	
Age					0.471
<65 years	21	17	10	10	
>65 years	18	8	12	8	
Gender					0.076
Female	8	13	7	6	
Male	31	12	15	12	
Localization of tumor					0.237
Right	3	4	8	4	
Lleft	33	21	14	14	
Histological grade					0.41
Well	1	0	1	2	
Moderate	29	19	17	15	
Poor	9	6	4	1	
TNM					0.041
1	0	0	2	0	
2	13	6	5	5	
3	24	13	11	6	
4	2	6	4	7	
Metastasis					0.012
No	37	19	19	11	
Yes	2	6	3	7	
Lymph node stage					0.738
0	14	7	7	8	
1	8	8	6	6	
2	17	10	9	4	
Microscopic tumor extension/T stage					0.792
2	1	1	2	1	
3	24	14	10	12	
4	14	10	10	5	
Lymphovascular invasion					0.366
No	13	5	9	4	
Yes	26	20	13	14	
Perineural invasion					0.919
No	14	9	9	8	
Yes	25	16	13	10	
KRAS					0.028
Wild	28	12	10	6	
Mutant	11	13	12	12	
NRAS					0.022
Wild	32	25	21	18	
Mutant	7	0	1	0	
BRAF					0.519
Wild	28	25	22	17	
Mutant	1	0	0	1	

SIRT1: Sirtuin 1; SIRT2: Sirtuin 2; TNM: Tumor, node, metastasis staging system; K-RAS: Kirsten rat sarcoma viral oncogene homolog; N-RAS: Neuroblastoma RAS viral (v-ras) oncogene homolog; B-RAF: B-Raf proto-oncogene, serine/threonine kinase. Categorical data were analyzed using the Chi-Square test or Fisher's Exact test, as appropriate.

Additional studies are required to elucidate the mechanisms within the KRAS signaling pathway that could inform potential treatment strategies. Notably, while the relationship between sirtuins and KRAS mutations has been minimally explored in other cancers, there is currently no literature addressing this association in colorectal cancer, making it a particularly intriguing area for further investigation. Teasley et al.[25] explored the relationship between K-RAS and SIRT1 in patients with endometrial and ovarian cancers, finding that nuclear SIRT1 expression was associated with KRAS expression in endometriosis-associated ovarian cancers. Similarly, Cheng et al.,[26] in a study on tobacco exposure and lung cancer, reported that increased SIRT1 expression due to tobacco exposure activates the downstream ERK1/2 pathway by enhancing K-Ras deacetylation via SIRT1, promoting the conversion of Ras-GTP to Ras-GDP.

Therapeutic potential for SIRT2 has recently gained attention through these mechanisms.[27] Yang et al.[28] demonstrated that SIRT2 could serve as a therapeutic target in KRAS mutant cancers. Studies on this topic include both *in vitro* and animal research. Bajpe et al.[29] showed that SIRT2 inhibition reduced drug resistance in KRAS mutant colorectal cancers *in vitro*; however, these findings were not replicated *in vivo*. The authors attributed this to the use of various chemotherapeutics by patients, which may interact with sirtuin mechanisms, and potential changes in sirtuin isoforms *in vivo*, altering their influence on drug resistance.

In a rat study, Song et al.[30] demonstrated that SIRT2 deletion increased KRAS-induced tumorigenesis. In this context, our study adds to the literature by demonstrating a relationship between SIRT1, SIRT2, and KRAS in colorectal cancers. Future research should involve an integrated analysis of multiple SIRT members across diverse CRC models and investigate the roles of these proteins at different stages of CRC progression, including pre- and post-metastasis.

Our study faced several limitations that should be acknowledged. First, the sample size and case selection were notable constraints. The limited inclusion of early-stage tumors, with only two cases classified as stage I, significantly impacted the study's statistical power and its ability to generalize findings across all stages of colorectal cancer. This imbalance may have limited our ability to draw robust conclusions regarding the prognostic role of sirtuins in the early stages of the disease. Second, the retrospective nature of the study presents inherent limitations. Retrospective designs are susceptible to biases related to data availability and selection, which can affect the reliability of observed associations. Moreover, the absence of survival analysis in our study further limits its ability to provide direct insights into the prognostic relevance of sirtuins in terms of patient outcomes, such as overall or disease-free survival. Third, the study relied solely on the IHC, which, while widely used and informative, has its limitations in providing quantitative or mechanistic insights into protein expression and function. Additionally, the use of existing tissue samples obtained from paraffin-embedded blocks may have affected the quality and consistency of staining, potentially introducing variability in the assessment of SIRT1 and SIRT2 expression. Addressing these limitations in future studies will be essential to validate and expand upon these findings.

CONCLUSION

SIRT2 expression was significantly associated with TNM stage and the presence of metastases, while SIRT1 expression did not show such correlations. Neither marker was related to vascular or perineural invasion, lymph node involvement, or microscopic tumor spread. Importantly, co-expression of SIRT1 and SIRT2 demonstrated significant associations with TNM stage, metastasis, and KRAS mutation status, suggesting that their combined evaluation may provide additional prognostic value. These findings highlight the potential roles of SIRT1 and SIRT2 as diagnostic and prognostic biomarkers in colorectal cancer, although validation in larger patient cohorts is warranted.

Ethics Committee Approval: The SBÜ Kartal Dr. Lütfi Kırdar City Hospital Clinical Research Ethics Committee granted approval for this study (date: 28.04.2021, number: 2021/514/200/11).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Conflict of Interest: The authors have no conflict of interest to declare.

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