Original paper

Clinicopathological importance of immunohistochemical expression of OCT4, c-MYC and Ki-67 in colorectal cancer

Rabia Hursitoglu[1], Abdulkadir Yasir Bahar[1], Sezen Koçarslan[1], Gökmen Aktaş[2], Neslihan Kurtul[3], Emine Kilinç[1]

[1]Department of Pathology, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey
[2]Department of Oncology, Kahramanmaraş Sütçü İmam University School of Medicine, Kahramanmaras, Turkey
[3]Department of Radiation Oncology, Kahramanmaraş Sütçü İmam University School of Medicine, Kahramanmaras, Turkey

Cancer stem cells (CSCs) are cancer cells responsible for cancer initiation, growth, metastasis, recurrence and resistance to treatment. OCT4 and c-MYC are widely accepted as CSC markers.

In this study, we examined the immunohistochemical co-expression of c-MYC and OCT4 with Ki-67 in colorectal cancers (CRC) and the relationship between the results and prognostic and therapeutic data. c-MYC, OCT4 and Ki67 immunohistochemical staining was applied to 162 colectomy cases. Nuclear staining was considered for immunohistochemical staining. Survival in the c-MYC^{H}/OCT4^{H} subtype, which is one of the subtypes based on c-MYC and OCT4 co-expression, was different from the other subtypes and statistically significant. Although these markers are enriched in cancer stem cells, their specificity in identifying them is limited. CSCs become dormant in the cell cycle, which is one of the mechanisms of escape in drug resistance. We hypothesized that including Ki67 immunohistochemical staining in our study would increase the specificity in detecting CSCs.

Our results show that the Ki67^{L}/c-MYC^{H}/OCT4^{H} subgroup was associated with lower survival and resistance to treatment compared to the other subgroups. This finding may provide insight into cases with a high number of CSCs and guide targeted treatments.

Key words: colorectal cancer, c-MYC, immunohistochemistry, Ki67, prognosis, therapy.

Introduction

Colorectal cancer (CRC) comprises 10.2% of total malignancies (1.85 million new cases/year), is the third most frequent malignancy in the world in general, and is the second most frequent cause of cancer-related death [1]. Targeted treatment and immunotherapy have assisted in structuring the patient selection criteria for CRC treatment and provided an increase in long-term and progression-free survival for patient groups with different molecular properties [2]. However, treatment failure, tumor recurrence and low patient survival (5-year survival rate nearly 50%) continue to be problems [3].

As a result of genetic and epigenetic changes and microenvironmental differences, cancer cells are heterogeneous even within the tumor of one individual [4]. The hierarchical cancer model proposes the presence

of a small subpopulation of high-degree tumorigenic cancer stem cells (CSCs) within a tumor. These CSCs have similar function to embryonic stem cells and have the capacity for uncontrolled growth, self-renewal and pluripotency [5–7].

CSCs are accepted as one of the main causes limiting treatment of cancers, including CRC [8, 9]. The presence of CSCs in a large portion of the tumor may lead to the tumor resisting traditional chemotherapies and cause recurrence and metastasis [10]. There is a need for biomarkers for risk classification and to predict therapeutic benefit by identifying CSCs in cancers. Additionally, identifying and destroying CSCs may be an effective treatment method [11]. While many markers are used to profile CSCs, the markers OCT4, NANOG, SOX2, KLF4 and c-MYC, associated with embryonic stem cells especially, are used to identify CSCs in solid tumors [5, 12–14].

Protooncogene c-MYC is a member of the MYC protein family involved in many biological processes, including the cell cycle, cell differentiation and protein synthesis [15, 16]. The c-MYC gene regulates both oncogenes and tumor-suppressing genes, and for this reason is known to play an important role in the emergence and development of cancer [17]. c-MYC is a cancer stem cell marker. A study by Martini *et al*. [18] found that high c-MYC expression rapidly advanced in untreated CRC cells and CRC metastasis displayed higher c-MYC expression than the primary tumor. Similar studies associated high c-MYC expression with low survival in CRC patients [19, 20]. Other studies contrarily reported an association with positive prognosis [21–23]. When these studies are assessed together, the prognostic value of c-MYC is controversial for CRC [24].

Ki-67 is a labile, non-histone nuclear protein that may be expressed in the G1, S, G2 and M phases of the cell cycle. It is catabolized at the end of the M phase and cannot be identified in G0 and early G1 cells [25]. In cancer, cells with high Ki-67 expression have a higher degree of differentiation and maturation and as a result Ki-67 is lower in more immature and less differentiated cancer stem cells [26]. In immobile situations, CSCs (in the G0 cell cycle phase) are resistant to traditional anticancer medications that become effective with progression of the cell cycle [27]. Ki-67 expression is a tumor marker used for a long time in clinical practice; however, its association with prognosis is controversial [28, 29].

OCT4 is an important member of the POU transcription factors required for self-renewal features and differentiation potential of pluripotent embryonic stem and germ cells [30]. Recent studies found that cells expressing high OCT4 levels were present in most solid carcinomas and that it was associated with worse prognosis [31, 32].

In our study, the importance of immunohistochemical Ki-67, OCT4 and c-MYC co-expression in cancer tissue from CRC cases and correlations with clinicopathological data, survival and cytotoxic treatments were investigated. It is hypothesized that combining the OCT4, c-MYC and Ki-67 immunohistochemical markers will provide higher accuracy for the detection of cancer stem cells and resolve the contradictory results about chemo-radiotherapy resistance in colorectal cancer and the associations of c-MYC and Ki-67 with prognosis.

Material and methods

Selection of tissue samples

The study included 162 cases with colectomy performed during 2009–2019 from the Department of Pathology archives of Kahramanmaraş Sütçü İmam University Research and Application Hospital in Türkiye. Nine cases were removed from the study due to inadequate data/material or death linked to early complications developing after surgical treatment. Investigation of cases was conducted by two pathologists based on a blinded experiment. Histological grading and histological subtyping of tumors were performed according to the WHO classification, 5th edition. Cancer stage was determined based on the American Joint Cancer Committee on Cancer, 8th edition. Clinical, pathological and survival data for patients were obtained from hospital electronic records.

Immunohistochemistry

After selecting paraffin blocks representing the tumor in CRC cases, immunohistochemical staining protocols were applied with a Ventana automatic staining device Benchmark XT (Ventana Medical Systems, Roche, USA) using anti-Ki-67 (30-9) Rabbit Monoclonal Primary Antibody, anti-c-MYC (Y69) Rabbit Monoclonal Primary Antibody, and OCT4 (MRQ-10) Mouse Monoclonal Antibody.

With c-MYC, slightly isolated nuclear positive staining was observed in normal mucosa. For statistical analysis, any intensity of c-MYC nuclear staining in more than 10% of neoplastic cells was scored as high [22]. Carcinoma cells with Ki-67 nuclear immunoreactivity were accepted as high-expressing cells, and the percentage was assessed. In ROC curve analysis for survival, 40% was chosen as the cut-off for the highest specificity and sensitivity. OCT4 expression was observed in colon epithelium basal cells. Nuclear staining in tumor tissue for OCT4 was noted, and scoring used a previously modified scoring system for both intensity and percentage of tumor cells [33]. In short, the positivity degree was scored as 0 when high-expressing cells were not observed; a score of 1 was given when the high-expressing

cell percentage was < 10%; a score of 2 was given for a percentage of 10–50%; and a score of 3 was given for a percentage > 50%. If high cells were not identified, intensity was scored as 0, with scores of 1 for weak staining, 2 for moderate staining and 3 for strong staining. Multiplying the scores for intensity and high-expressing cell percentage gave immunohistochemical staining degrees of 0, 1, 2, 3, 4, 6 and 9. For statistical analysis, cases with staining degrees of 0, 1 and 2 were accepted as unstained and given 0 points. Cases with staining degrees of 3 and 4 were accepted as moderately stained and given 1 point. Cases with staining degrees of 6 and 9 were accepted as strong staining and given 2 points.

Statistical analysis

All statistical analyses were performed with SPSS version 20 (IBM, Armonk, NY, USA). The χ^2 test was used to assess the correlations between clinicopathological features with c-MYC, OCT4 and Ki-67 expression. Survival probabilities were predicted with the Kaplan-Meier method and probabilities compared with the log-rank method. Multivariate analysis used the Cox regression test to research the effect of prognostic factors on each other. $P < 0.05$ was accepted as statistically significant.

Results

Clinicopathological descriptive findings

Histological subtype assessment revealed 133 cases (86.9%) of conventional adenocarcinoma, 16 cases (10.5%) of mucinous adenocarcinoma, 3 cases (2%) of signet ring cell carcinoma and 1 case (0.7%) of micropapillary adenocarcinoma.

When assessed according to the 2017 American Joint Cancer Committee TNM classification, 18 cases (11.8%) were stage 1, 61 cases (39.9%) were stage 2, 54 cases (31.11%) were stage 3 and 20 cases (13.1%) were stage 4. During follow-up, 3 cases in stage 1, 16 cases in stage 2, 14 cases in stage 3 and 15 cases in stage 4 died.

One patient (0.7%) had chemotherapy before surgical treatment, 78 patients (51.0%) had chemotherapy after surgical treatment and 19 cases (12.4%) had chemotherapy before and after surgical treatment. Thirty-six cases (23.5%) received radiotherapy. Eighteen of 55 patients without treatment and 30 of 98 patients receiving cytotoxic treatment with chemo- and radiotherapy died.

Clinicopathological effects of OCT-4 expression in CRC patients

OCT4 expression was assessed as high in 42 cases (27.45%) and low for 111 cases (72.54%) (Fig. 1). The correlations between clinicohistopathological data with OCT4 expression are given in Table I. Significant correlations were found between high OCT4 expression and age < 65 years, histological type and high histological grade ($p = 0.008, p = 0.011, p = 0.029$, respectively) (Table I).

The mean survival duration in the group with high OCT4 expression was 72.5 ± 9.3 months, while mean survival in the group with low OCT4 expression was 96.2 ± 5.7 months. For overall survival, there was no statistically significant correlation with OCT4 expression ($p = 0.069$) (Fig. 2).

For those receiving cytotoxic treatment with chemo- and radiotherapy, mean survival duration for OCT4 high cases was 79.5 ± 9.6 months, while it was 87.3 ± 7.2 months for OCT4 low cases. For those not receiving treatment, mean survival duration was 49.4 ± 16.1 months for OCT4 high cases and 105.1 ± 8.2 months for OCT4 low cases. There was no significant association between OCT4 expression and survival for those receiving cytotoxic treatment with chemo- and radiotherapy, while there was a significant association between OCT4 expression and survival for those not receiving treatment ($p = 0.0001$).

Clinicopathological effects of c-MYC expression in CRC patients

c-MYC expression was assessed as high in 66 cases (43.13%) and low in 87 cases (56.86%) (Fig. 1). The correlations between clinicohistopathological data and c-MYC expression are given in Table I. Significant correlations were found between high c-MYC expression and both smaller tumor size and lower histological grade ($p = 0.033, p = 0.012$, respectively).

The mean survival duration in the group high for c-MYC expression was 95.5 ± 7.9 months, while the mean survival in the group low for c-MYC expression was 82.08 ± 5.7 months. For overall survival, there was no statistically significant correlation between survival and c-MYC expression ($p = 0.522$).

Among those with cytotoxic treatment with chemo- and radiotherapy, mean survival of c-MYC high cases was 103.2 ± 2.2 months, while mean survival of c-MYC low cases was 73.4 ± 6.5 months. For those without treatment, mean survival for c-MYC high cases was 83.2 ± 11.8 months, while mean survival of c-MYC low cases was 93.04 ± 9.6 months. No statistically significant correlation was found between c-MYC, treatment and survival ($p = 0.280$) (Fig. 3).

Clinicopathological effects of Ki-67 expression in CRC patients

Ki-67 expression was < 40% in 89 cases (58.16%) (Fig. 1) and ≥ 40% (high) in 64 cases (41.83%). The correlations between clinicohistopathological data and Ki-67 expression are given in the Table I. There was a significant correlation between cases with

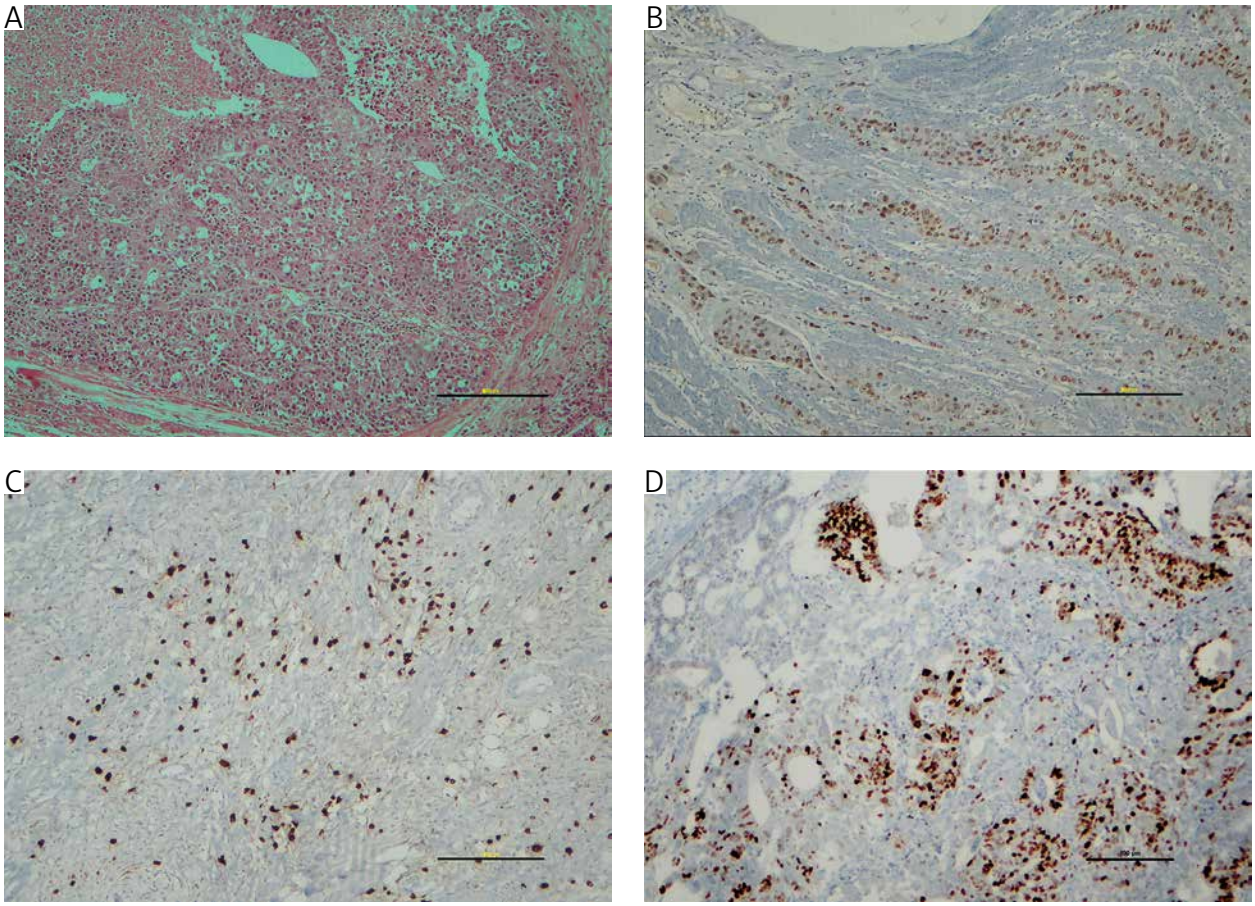

Fig. 1. A) Histologically high-grade colorectal carcinoma (hematoxylin and eosin, 20× magnification). **B**) c-Myc nuclear stain detected in small tumor nests and single cells (immunohistochemistry, 20× magnification). **C**) OCT-4 positivity in tumor buds and singly infiltrating tumor cells (immunohistochemistry, 20× magnification). **D**) Histologically high-grade tumor with low Ki67 proliferation score (immunohistochemistry, 20× magnification)

< 40% Ki-67 expression and distant metastasis ($p = 0.034$) (Table I).

The mean survival duration was 76.2 ± 6.05 months in the group with < 40% Ki-67 expression, while it was 106.9 ± 7.6 months in the group with ≥ 40% (high) Ki-67 expression. For overall survival, there was a statistically significant correlation with Ki-67 expression ($p = 0.005$) (Fig. 4).

For those receiving cytotoxic treatment with chemo- and radiotherapy, mean survival of < 40% Ki-67 expression cases was 74.6 ± 7.8 months, while it was 102.7 ± 8.9 months for ≥ 40% (high) Ki-67 expression cases. For those not receiving treatment, mean survival for those with < 40% Ki-67 expression was 75.5 ± 9 months, while mean survival for those with ≥ 40% (high) Ki-67 expression was 109.6 ± 10.5 months.

There was no significant association between Ki-67 expression and survival for those not receiving treatment, while there was a significant correlation between cytotoxic treatment with chemo- and radiotherapy and < 40% Ki-67 expression and low survival ($p = 0.012$).

c-MYC and OCT4 co-expression in CRC patients

Four subtypes were created according to c-MYC and OCT4 co-expression: c-MYC^{L}/OCT4^{L} ($n = 50$), c-MYC^{H}/OCT4^{L} ($n = 61$), c-MYC^{L}/OCT4^{H} ($n = 26$) and c-MYC^{H}/OCT4^{H} ($n = 16$).

The longest mean survival was observed in the c-MYC^{H}/OCT4^{L} (106.8 ± 8.01) subtype, with the shortest mean survival in the c-MYC^{H}/OCT4^{H} (34.7 ± 6.9) subtype. The survival in the c-MYC^{H}/OCT4^{H} subtype was different from the other subtypes, and this was statistically significant ($p = 0.004$) (Fig. 5).

For those not receiving treatment, the c-MYC^{H}/OCT4^{L} subtype had the longest mean survival duration (100.3 ± 11.6), while the c-MYC^{H}/OCT4^{H} subtype had the shortest mean survival (8.0 ± 6.02). For those not receiving treatment, the c-MYC^{H}/OCT4^{H} subtype had different survival to the other subtypes, and this was statistically significant ($p = 0.0001$) (Fig. 7).

For cases receiving cytotoxic treatment with chemo- and radiotherapy, no statistically significant difference was observed for survival (Fig. 7).

Table I. Relationship between clinicopathological findings and c-MYC, OCT4 and Ki-67 expression

Factor		cMYC			OCT-4			Ki-67		
		Negative	Positive	*p*-value	Negative	Positive	*p*-value	< 40%	≥ 40%	*p*-value
Age	≥ 65	48/85	37/85	0.913	69/85	16/85	0.008	49/85	36/85	0.883
	< 65	39/68	29/68		42/68	26/68		40/68	28/68	
Gender	Male	58/101	43/101	0.845	74/101	27/101	0.781	58/101	43/101	0.795
	Female	29/52	23/52		37/52	15/52		31/52	21/52	
Tumor size	≥ 5	52/80	28/80	0.033	60/80	20/80	0.477	51/80	29/80	0.143
	< 5	35/73	38/73		51/73	22/73		38/73	35/73	
Localization	Right	39/59	20/59	0.059	40/59	19/59	0.549	31/59	28/59	0.521
	Left	25/56	31/56		43/56	13/56		34/56	22/56	
	Rectum	23/38	15/38		28/38	10/38		24/38	14/38	
Grade	Low	60/117	57/117	0.012	90/117	27/117	0.029	70/117	47/117	0.453
	High	27/36	9/36		21/36	15/36		19/36	17/36	
Histological type	Adenocarcinoma NOS	72/133	61/133	0.331	101/133	32/133	0.011	78/133	55/133	0.739
	Mucinous Adenocarcinoma	12/16	4/16		9/16	7/16		8/16	8716	
	Signet ring cell carcinoma	2/3	1/3		0/3	3/3		2/3	1/3	
	Micropapillary adenocarcinoma	1/1	0/1		1/1	0/1		1/1	0/1	
Stage	1	8/18	10/18	0.520	14/18	4/18	0.956	8/18	10/18	0.140
	2	33/61	28/61		44/61	17/61		35/61	26/61	
	3	33/54	21/54		39//54	15/54		30/54	24/54	
	4	13/20	7/20		14/20	6/20		16/20	4/20	
pN	0	48/89	41/89	0.689	65/89	24/89	0.941	52/89	37/89	0.219
	1	25/41	16/41		30/41	11/41		27/41	14/41	
	2	14/23	9/23		16/23	7/23		10/23	13/23	
pM	0	74/133	59/133	0.431	97/133	36/133	0.784	73/133	60/133	0.034
	1	13/20	7/20		14/20	6/20		16/20	4/20	
Lympho-vascular invasion	Positive	24/46	22/46	0.443	35/46	11/46	0.520	29/46	17/46	0.423
	Negative	63/107	44/107		76/107	31/107		60/107	47/107	
Perineural invasion	Positive	16/32	16/32	0.378	20/32	12/32	0.152	23/32	9/32	0.077
	Negative	71/121	50/121		91/121	30/121		66/121	55/121	
Cytotoxic therapy	Yes	60/98	38/98	0.146	69/98	29/98	0.428	60/98	38/98	0.307
	No	27/55	28/55		42/55	13/55		29/55	26/55	

For c-MYC and OCT4 high co-expression, the percentage with a high histologic grade was 25%, while it was 10% in the double low group, and the difference was statistically significant ($p = 0.015$).

In the c-MYC^{H}/OCT4^{H} subtype, the mucinous histological type was not observed, and there was a statistically significant difference compared to the other subtypes ($p = 0.017$).

Correlation of c-MYC, OCT4 and Ki-67 co-expression with survival in CRC cases

When the significant correlation of survival for cases high for both c-MYC and OCT4 expression is assessed by adding the Ki-67 expression status, a significant correlation was found for Ki67^{L}/c-MYC^{H}/OCT4^{H} cases with survival ($p = 0.0001$) (Fig. 6).

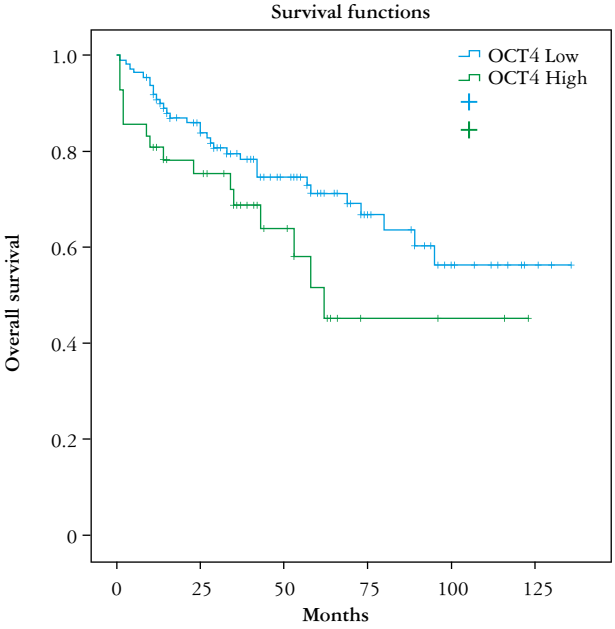

Fig. 2. Kaplan-Meier curves representing survival rate depending on the Oct4+ cells proportion

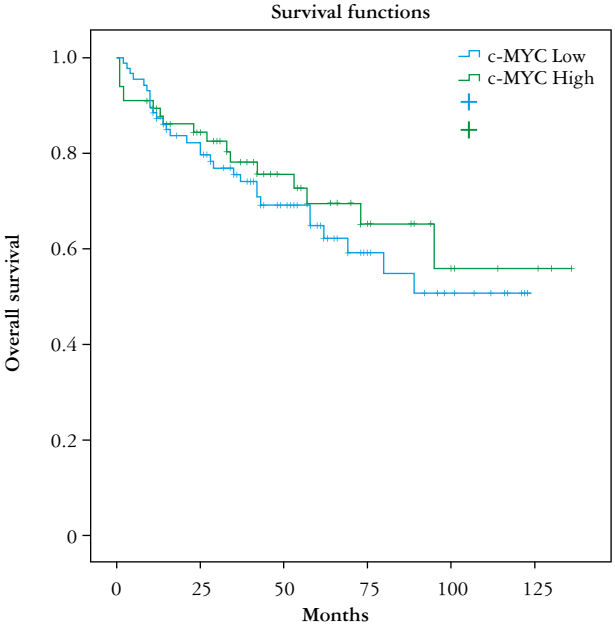

Fig. 3. Kaplan-Meier curves representing survival rate depending on the c-MYC+ cells proportion

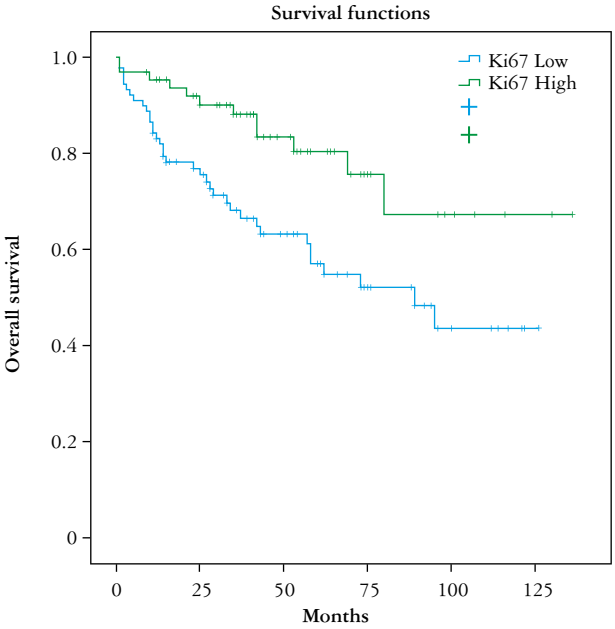

Fig. 4. Kaplan-Meier curves representing survival rate depending on the Ki67+ cells proportion

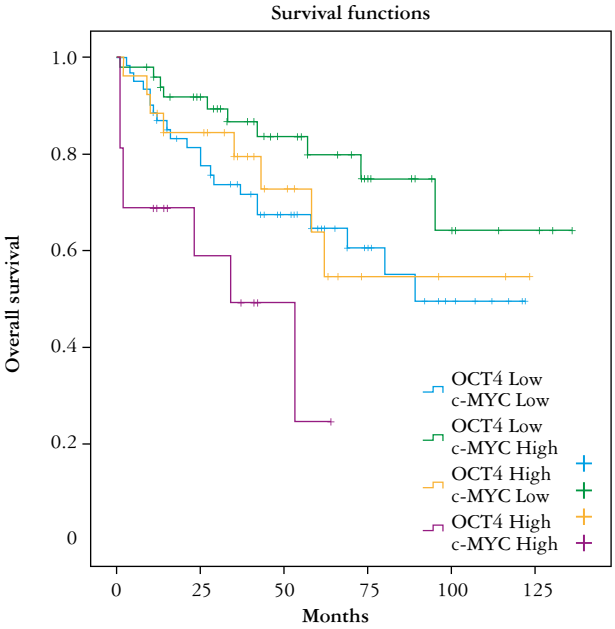

Fig. 5. Kaplan-Meier curves representing survival rate depending on the combination of OCT4+ and c-MYC+ cells proportions

Cox regression multivariate analysis identified advanced age, stage and Ki-67^{L}/c-MYC^{H}/OCT4^{H} subtype as independent prognostic factors.

Among these prognostic factors, the Ki-67^{L}/c-MYC^{H}/OCT4^{H} subtype appears to be a more valuable factor than the others (Table II).

Discussion

The cancer stem cell concept is supported by increasing numbers of studies showing the presence of CSC subpopulations expressing stem cell markers. Additionally, CSCs are an undiscovered area of research with the potential to develop new treatments for very diverse cancers.

OCT4 and c-MYC are stem cell markers, key factors in inducing pluripotentiality of somatic cells, and are used to identify cancer stem cell subpopulations in a range of cancer types. As a result, in our study, the aim was to reveal CSC medication resistance with these markers and hence to identify CSCs responsible for low survival and to reveal the profile

of patients to whom targeted treatment can be applied.

In the literature, several studies have focused on the correlation between c-MYC and CRC prognosis, though the results are inconsistent. In CRC patients, c-MYC expression is significantly associated with poor survival. While there are studies reporting that c-MYC expression is an independent poor prognosis factor, there are also studies reporting that it is associated with good prognosis in CRC patients [19–22]. In our study, patients with high c-MYC expression had mean survival duration of 95.5 ± 7.9 months, while patients with low c-MYC expression had mean survival of 82.08 ± 5.7 months. There was no statistically significant correlation between survival and c-MYC expression.

In the literature, different results are reported for the relationship between OCT4 and CRC prognosis. A study by Voutsadakis et al. about the effect of OCT4 on prognosis showed that OCT4 expression was associated with poor prognosis in CRC patients [34]. Another study by Dai et al. found that OCT4 was beneficial for inhibition of colon cancer cells and protection against metastasis [35]. Additionally, a study that assessed OCT4 expression comparing polyp and cancer cases with healthy controls found no significant correlation [36]. In our study, the mean survival duration for patients with high OCT4 expression was 72.5 ± 9.3 months, while for patients with low OCT4 expression mean survival was 96.2 ± 5.7 months. However, no statistically significant correlation was found between survival and OCT4 expression.

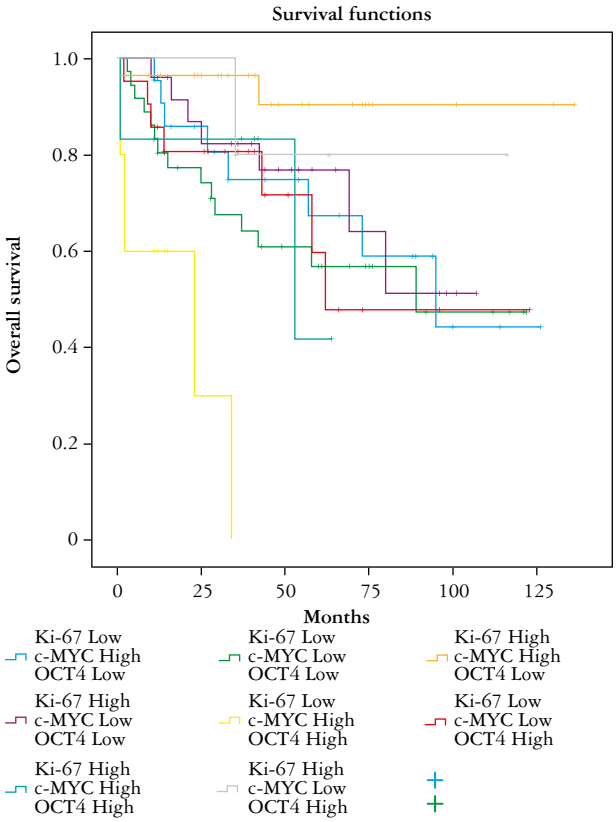

Fig. 6. Kaplan-Meier curves representing survival rate depending on the combination of OCT4+, Ki67+ and c-MYC+ cells proportions

A study related to the role of OCT4 in CSC and chemotherapy resistance by Gwak *et al.* [37] found that high immunohistochemical OCT4 expression

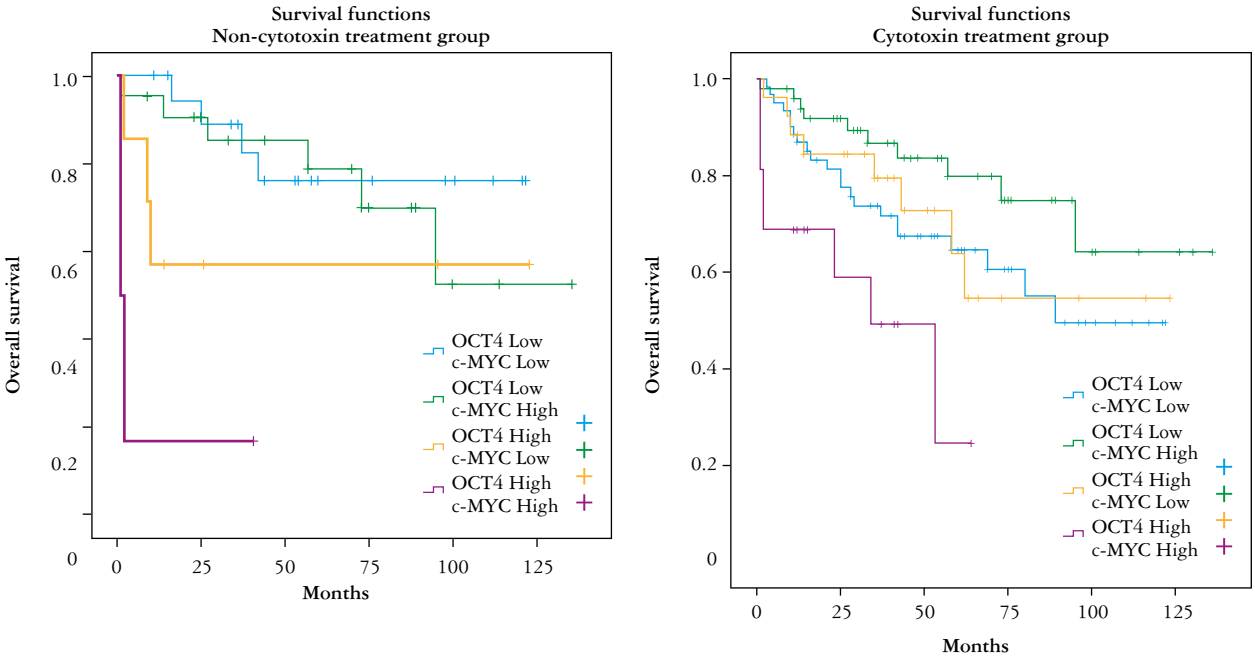

Fig. 7. Kaplan-Meier curves representing survival rate depending on the combination of OCT4+ and c-MYC+ cells proportions according to the of cytotoxic therapy

Table II. Multivariate analysis

	Hazard ratio	*p*-value	95% confidence interval
Advanced age (> 65)	1.75	0.094	0.304–1.098
Advanced stage (stage 3–4)	7.09	0.001	0.058–0.344
Ki-67L/c-MYCH/OCT4H	20.703	0.008	2.191–195.578

was associated with tamoxifen resistance in addition to poor clinical outcomes in breast cancer patients.

Additionally, Cheng *et al*. [38] found high levels of expression of the pluripotent transcription factors OCT4 and c-MYC in drug-resistant tumor cells and elevated Stat3 phosphorylation in a study of triple negative breast cancers.

In our study, to increase the sensitivity for detecting CSC populations in CRC tissue, a combined analysis of c-MYC and OCT4 expression was completed. As the prognostic value of c-MYC is controversial in CRC, it is necessary to support c-MYC with another CSC marker for both proliferation and synthesis in undifferentiated (stem cell) cells [39, 40]. Among the 4 subgroups created based on c-MYC and OCT4 co-expression, cases with c-MYC^{H}/OCT-4^{H} high co-expression were found to have lowest survival (34.7 ± 6.9), and this was statistically significantly different than the other co-expression subtypes ($p = 0.004$).

Studies of CRC cases obtained inconsistent results for the correlation between Ki-67 expression and patient prognosis [41]. Shin *et al.* [42] found that Ki-67 overexpression was associated with poor prognosis in CRC. A meta-analysis study of patients with colorectal adenocarcinoma reported that the proliferation index may be used as a prognostic marker, especially for metastasis associated with poor prognosis and left colon tumors [42]. Another study by Reimers *et al*. [43] investigated Ki-67 and caspase-3 tumor expression in stage I-IV colon cancer patients. They observed the best prognosis in patients with both high apoptosis and proliferation levels. A study by Melling *et al*. [44] showed that immunohistochemical high Ki-67 expression in CRC was an independent positive prognostic marker. Our study corroborates the study by Melling and Reimers and a statistically significant correlation was found between low Ki-67 < 40% (cut-off value determined with ROC analysis) expression and distant metastasis ($p = 0.034$) and low survival ($p = 0.005$). In our study, low survival was observed in the Ki-67 expression subgroup with < 40% expression and receiving cytotoxic treatment with chemo- and radiotherapy.

One of the self-protection mechanisms of normal stem cells is remaining in the G0 phase most of the time [45, 46]. The definite mechanisms of resistance against radiation and chemotherapeutic drugs of CSC, sharing several of the features of normal stem cells, are still not fully understood; however, several potential views have been discussed. One of the important ideas is that CSC do not enter the proliferation cycle. They may remain silent in the G0 phase and as a result may be resistant to radiotherapy and chemotherapy [47]. Several studies in the literature used the Ki-67 protein more or only the proliferation index. Our aim in using this protein in our study was to support the hypothesis that low Ki-67 in cells high for CSC markers would assist in identifying cancer stem cells in the G0 phase.

There are contradictory results about the relationship between independent c-MYC and Ki-67 expression with survival in studies in the literature. In our study, we did not find a significant correlation between c-MYC independent expression and survival. The reason for these contradictory results may be that c-MYC is expressed by a heterogeneous cell group – apart from stem cells. Similarly, patient series in studies assessing Ki-67 expression may have caused more or less contradictory results for cases with low Ki-67 and high CSC potential. We assumed that combining the OCT4, c-MYC and Ki-67 immunohistochemical markers would allow us to explain these contradictions. When the OCT4, c-MYC and Ki-67 immunohistochemical markers are combined, the shortest survival duration was in the Ki-67^{L}/c-MYC^{H}/OCT4^{H} subgroup, and this was statistically significantly different from the other subgroups ($p = 0.0001$).

Additionally, while mean survival in the group receiving cytotoxic treatment with chemo- and radiotherapy was 86.9 ± 6.2 months, the lowest survival of 28.5 ± 5.5 months was for the Ki-67^{L}/c-MYC^{H}/OCT4^{H} subgroup ($p = 0.047$). This suggests that this group is more resistant to treatment. This finding may provide an idea about identifying cases with high CSC responsible for resistance to treatment in CRC and low survival.

To the best of our knowledge, though there are studies related to c-MYC, OCT4 and Ki-67 in CRC, there is no study assessing the correlation of the combined form of these parameters with prognosis and cytotoxic treatment with chemo- and radiotherapy.

In our study, the importance of the independent and combined immunohistochemical expression of Ki-67, OCT4 and c-MYC in tumor tissue of patients with colorectal cancer was evaluated along with the correlation with treatment response in patients treated with chemotherapy and radiotherapy. Additionally, in our study, 20 cases (13.1%) were in stage 4. The low

number of cases in this group may limit assessment of the relationship between immunohistochemical parameters and metastasis. More CSC markers may be used; however, increasing the number of subgroups would have lowered the patient numbers in each group and made statistical assessment more difficult. The view that Ki-67 and c-MYC expression levels are heterogeneous was supported.

Our findings assessing cancer stem cells and proliferation of these cells show significant correlations of the Ki-67^{L}/c-MYC^{H}/OCT4^{H} subgroup with low survival and drug resistance, compared to the other groups. Although this result will contribute to understanding problems encountered with cancer treatment like cancer biology, distant metastasis, relapse and drug resistance, our results should be considered preliminary and should be supported by advanced studies.

Disclosures

1. The study received approval from the Research Ethics Committee of the KSU University Faculty of Medicine (Protocol number: 282).
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

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Address for correspondence:

Rabia Hursitoglu
Department of Pathology
Kahramanmaras Sutcu Imam University
Department of Pathology
Bati cevreyolu St. No: 251/A 46040
Kahramanmaras, Turkey
e-mail: rabiahursitoglu@gmail.com