The Predictive Value of hsa_circ_0001313 (circCCDC66) in Egyptian Rectal Cancer Patients: A New Era in Precision Medicine

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Abstract

Introduction The involvement of circular RNAs (circRNAs) in cancer research has been emphasized in recent years due to evidence of their involvement in malignancy pathogenesis. Yet, the involvement of circRNAs in the resistance to cancer treatment remains to be clarified. Circular RNA 0001313 (circ_0001313) has a distinct expression in different cancers, and it is overexpressed in rectal cancer; hence, it could be a promising non-invasive stable biomarker and a therapeutic target for rectal cancer. Yet, its predictive role has not been studied in Egyptian rectal cancer patients.

Objective To study the predictive value of circulating circ_0001313 (circ_CCDCC6) in assessing the response to neoadjuvant chemoradiotherapy (nCRT) in patients with rectal cancer and its relation to radiological and pathological response.

Materials and Methods The present study included 50 patients with locally advanced rectal cancer and 20 healthy subjects as controls. The analysis of the relative circ_CCDCC6 expression was performed using the real-time quantitative polymerase chain reaction (qPCR) method.

Results The circ_CCDCC6 was found to be significantly more expressed in rectal cancer patients compared with controls ($p < 0.001$). Moreover, its expression level was significantly higher in nonresponders to nCRT compared with responders ($p < 0.001$). Furthermore, a receiver operating characteristic (ROC) curve analysis was performed to evaluate the predictive performance of circulating circ_0001313; its sensitivity in predicting the response to treatment was of 93.33%, and its specificity was of 91.43%.

Conclusion Significant up-regulation of circ_0001313 in rectal cancer suggests a potential oncogenic role, and higher expression of circ_0001313 among nonresponders suggests that it could be a predictor of the response to nCRT.

Keywords
- circ_0001313
- circular RNA
- rectal cancer
- gene expression
- response to nCRT

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Introduction

Colorectal cancer (CRC) is the third most prevalent cancer worldwide and is responsible for the second highest number of cancer-related deaths globally.[1] Approximately 31% of all CRC cases are of rectal cancer.[2] Patients diagnosed with locally-advanced rectal cancer undergo neoadjuvant chemoradiotherapy (nCRT) as the primary treatment, followed by surgery, and nCRT improves the outcome of surgery, decreases the chances of local recurrence, and increases the rate of preservation of the sphincter.[3] Patient response to nCRT is variable, from complete pathological response to resistance in the form of stable and progressive disease.[4] This is due to the heterogeneity of the disease, meaning that standard treatments, like radiation or chemotherapy, may only work in a subset of patients.[5]

The molecular patterns of tumors vary from one patient to another. This variability has generated new opportunities in the field of precision and personalized medicine (PPM).[6] Therefore, there is a critical need for molecular biomarkers that can predict response to nCRT to identify the patients who will benefit from the treatment and those who will be resistant to it, avoiding unnecessary exposure to CRT, reducing its toxicity, and finding a better alternative for such patients.[7]

In this context, circular RNAs (circRNAs) are noncoding molecules that were found to be widely expressed in eukaryotes. They are highly stable RNA molecules without 5′ to 3′ tails: these tails are joined to form a covalently-closed continuous loop. This unique structure prevents degradation by exonucleases, and circRNAs have been successively detected in human peripheral whole blood, plasma, and other biological fluids, making them promising, non-invasive, stable biomarkers in different diseases.[8,9] The expression of circRNAs occurs in a tissue-specific manner, which explains their significant role in human malignancy. Although their exact roles are yet to be determined, their potential as disease biomarkers and novel therapeutic targets is promising.[10] They may also play a role in regulating pathways that affect drug receptivity.[11] While many researchers have investigated the prognostic and diagnostic potentials of circRNAs as tumor biomarkers, their role in the resistance to cancer treatment remains unclear.[11]

The circ_CCDC66, also known as circ_0001313, is a noncoding RNA that is 468 nucleotides long. It originates from the parental *CCDC66* gene. Studies have shown that it plays a role in the development of colon cancer, and its depletion can repress cell growth and trigger apoptosis in colon cancer cell lines. It has been suggested[12] that circ_0001313 modulates the radioresistance of CRC; however, its role as a potential circulating predictor of clinical and pathological responses to neoadjuvant treatment in patients with rectal cancer has not been explored yet. Accordingly, the current work aims to study the predictive value of circulating circ_0001313 in assessing the response of patients with rectal cancer to the neoadjuvant treatment.

Materials and Methods

Patients

The study involved 50 consecutive patients and 20 controls who were matched in terms of age and gender. The sample was composed of patients of both sexes aged between 18 and 75 years who had biopsy-proven rectal cancer. They were referred to the Clinical Oncology Department of Alexandria Main University Hospital and had diseases in stages II, III, or IVa according to the Tumor, Node, Metastasis (TNM) staging. All the participants provided written informed consent before being enrolled, and the study was conducted in accordance with the institutional protocols, following the Declaration of Helsinki, and it was approved by the Ethics Review Board of Alexandria University, Faculty of Medicine, under number 0201512.

Before starting nCRT, routine laboratory investigations were conducted, including a two-site sequential chemiluminescent immunometric assay to determine the serum levels of carcinoembryonic antigen (CEA) and an enzyme-linked immunosorbent assay (ELISA) to determine serum levels of carbohydrate antigen 19-9 (CA19-9). Then, the patients underwent the following nCRT protocol: preoperative radiation therapy at a dose of 45 GY in 25 fractions (1.8 GY per day) was delivered to the entire pelvis for 5 weeks, followed by a 5.4 GY boost in 3 fractions delivered to the primary tumor. Concurrent oral capecitabine was administered at a dose of 825 mg/m^2 twice a day during radiation therapy.[13] After 6 to 8 weeks following the neoadjuvant therapy, a noncontrast pelvic MRI scan was repeated and compared with the pretreatment pelvic MRI to assess the patient's radiological response to neoadjuvant therapy before referral to surgery. A combination of leucovorin calcium (folinic acid), fluorouracil, and oxaliplatin (FOLFOX) was considered as adjuvant chemotherapy for four cycles. Patient data, including age, sex, clinical presentation, treatments received, and follow-up results, were obtained.

Methods

Following the neoadjuvant treatment, a pathological examination of the rectosigmoidectomy specimens was performed for the studied patients. Subsequently, pathological grading of primary tumor regression was performed semiquantitatively through the determination of the amount of residual tumor cells compared with the desmoplastic response. Tumor regression grading (TRG) was conducted according to the American Joint Committee on Cancer (AJCC) classification as follows: TRG0–no residual tumor cells; TRG1–single cells or small groups of cells; TRG2 – residual cancer with desmoplastic response; and TRG3–minimal evidence of tumor response.[14] Before receiving nCRT, a venous blood sample was taken from the patients and the controls under strict aseptic conditions, and the plasma was separated for the determination of the expression of circ_0001313.

Determination of Plasma Circ_0001313 Expression Level Using Quantitative Reverse-Transcription Polymerase Chain Reaction (qRT-PCR)

Total RNA, including circRNA, was extracted from 200 µL of plasma according to the manufacturer's instructions of the miRNeasy Mini Kit (QIAGEN, Hilden, Germany). The concentration and purity of the RNA were then measured at 260, 280, and 230 nm using the Nano Drop 2000/2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States), in which ratios of A260/A280 and of A260/A230 of 1.8 to2.1 indicate highly-pure RNA.[15] Consequently, complementary DNA (cDNA) was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). Real-time polymerase chain reaction (PCR) was performed using the Maxima SYBR Green qPCR Master Mix (2X) (Thermo Fisher Scientific), and specific primers for circ_0001313: forward (*ACCTACAACCGGAAGCCAG*) and reverse (*AGCAGTACTGTTTCCTGATGC*). The PCR cycling conditions were as follows: initial denaturation of 95°C for 10 minutes, followed by 3-step cycling: denaturation (95°C, 15 seconds), annealing (65°C, 30 seconds), and extension (72°C, 30 seconds), and a melting-curve analysis was performed to verify specificity and identity of the PCR products. The relative expression of circ_0001313 was calculated using the comparative cycle threshold (CT) method (2–ΔΔCT) using glyceraldehyde-3- phosphate dehydrogenase (GAPDH) as the endogenous control.[16]

Statistical Analysis

The statistical analysis was performed using the Statistical Package Social Sciences for Windows (SPSS, IBM Corp., Armonk, NY, United States) software, version 20.0.

Results

Demographic and Laboratory Data

The patients were categorized into 4 groups according to the radiological response: group I – 9 patients with complete pathological response; group II – 26 patients with partial response; group III – 11 patients with stable disease; and group IV – 4 patients with progressive disease. There were no significant differences among the four groups regarding gender, age, and routine laboratory investigation (hemoglobin concentration [Hb conc] and CA19-9]) (►**Table 1**).

The level of CEA of groups II, III, and IV was significantly higher than that of the controls, and, regarding group I, it was significantly lower than that of groups II, III, and IV; however, there were no significant differences among groups II, III, and IV. An initial low CEA concentration (not exceeding 5.0

Table 1 Comparison of the studied groups according to demographic data and laboratory investigation in patient groups

	Complete response ($n=9$)		Partial response ($n=26$)		Stable disease ($n=11$)		Progressive disease ($n=4$)		Control ($n=20$)		Test of significance	*p*-value
	No.	%	No.	%	No.	%	No.	%	No.	%		
Sex												
Male	5	55.6	10	38.5	5	45.5	3	75.0	8	40.0	$\chi^2 = 2.515$	$^{MC}p = 0.677$
Female	4	44.4	16	61.5	6	54.5	1	25.0	12	60.0		
Age (years)												
Min.–max	40.0–68.0		41.0–79.0		43.0–71.0		40.0–59.0		32.0–67.0		F = 2.188	0.080
Mean ± SD	50.11 ± 11.79		54.15 ± 10.01		56.09 ± 9.54		49.25 ± 7.76		47.15 ± 8.73			
Median (IQR)	44.0 (40.0–62.0)		53.50 (46.0–60.0)		52.0 (50.50–64.0)		49.0 (44.50–54.0)		48.0 (41.50–52.50)			
Hb (%)												
Min–max	9.60–13.90		8.90–13.30		8.80–11.40		9.50–11.30		11.50–14.70		F = 25.659*	< 0.001*
Mean ± SD	10.76 ± 1.34		10.30 ± 1.14		10.03 ± 0.82		10.60 ± 0.81		13.17 ± 0.98			
Median (IQR)	10.40 (9.78–11.30)		10.15 (9.40–10.80)		10.10 (9.50–10.50)		10.80 (10.0–11.20)		12.95 (12.55–14.10)			
p_0	< 0.001*		< 0.001*		< 0.001*		< 0.001*					
Significance among groups	$p_1 = 0.792$; $p_2 = 0.550$; $p_3 = 0.999$; $p_4 = 0.957$; $p_5 = 0.985$; and $p_6 = 0.892$											
CEA												
Min–max	2.11–5.0		1.50–13.94		3.60–13.94		7.32–14.50		0.22–4.60		F = 21.168*	< 0.001*
Mean ± SD	3.57 ± 0.95		6.78 ± 3.17		8.69 ± 3.11		9.76 ± 3.22		1.81 ± 1.11			
Median (IQR)	3.32 (3.0–4.31)		6.82 (4.80–8.36)		8.36 (7.0–10.40)		8.61 (7.92–11.60)		1.79 (1.0–2.20)			

(*Continued*)

Table 1 (*Continued*)

	Complete response ($n=9$)		Partial response ($n=26$)		Stable disease ($n=11$)		Progressive disease ($n=4$)		Control ($n=20$)		Test of significance	*p*-value
	No.	%	No.	%	No.	%	No.	%	No.	%		
p_0	0.409		$< 0.001^*$		$< 0.001^*$		$< 0.001^*$					
Significance among groups	$p_1 = 0.013^*$; $p_2 < 0.001^*$; $p_3 = 0.001^*$; $p_4 = 0.227$; $p_5 = 0.189$; and $p_6 = 0.949$											
CA19-9												
Min–max	10.05–45.0		16.60–42.70		19.45–40.60		21.86–40.60		2.32–20.14		$F = 29.548^*$	$< 0.001^*$
Mean ± SD.	26.52 ± 11.29		28.55 ± 7.97		31.02 ± 7.09		28.81 ± 8.80		7.16 ± 4.44			
Median (IQR)	24.62 (20.50–30.73)		27.33 (21.75–35.0)		30.73 (27.12–37.20)		26.39 (22.08–35.53)		6.10 (3.85–8.76)			
p_0	$< 0.001^*$		$< 0.001^*$		$< 0.001^*$		$< 0.001^*$					
Significance among groups	$p_1 = 0.957$; $p_2 = 0.678$; $p_3 = 0.987$; $p_4 = 0.893$; $p_5 = 1.000$; and $p_6 = 0.987$											

Abbreviations: χ^2, Chi-squared test; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; F, one-way analysis of variance; Hb, hemoglobin; IQR, interquartile range; max, maximum; ^{MC}p, Monte Carlo *p*-value; min, minimum; SD, standard deviation.
Notes: One-way analysis of variance: pairwise comparisons between pairs of groups were performed using the Tukey Post-Hoc Test Monte Carlo *p*-value: *p*-value for the comparison of the four studied groups – p_1: value for the comparison between complete and partial responses; p_2: value for the comparison between complete response and stable disease; p_3: value for the comparison between complete response and progressive disease; p_4: value for the comparison between partial response and stable disease; p_5: value for the comparing between partial response and progressive disease; and p_6: value for the comparison between stable and progressive diseases. *Statistically significant at $p \leq 0.05$.

ng/mL) may be one of the factors associated with complete clinical and pathological response (►**Table 1**).

Plasma Level of Circ¬_0001313 and Its Relationship with Radiological Response

The level of plasma expression of circ_0001313 of rectal cancer patients was significantly higher (median: 3.72; range: 1.17–45.25) than that of the controls (median: 1.06; range: 0.62–1.46) ($p < 0.001$). The diagnostic performance of the plasma expression of circ_0001313 is shown in (►**Fig. 1**). At the cut-off value of > 1.2397 fold change, the sensitivity of the plasma expression of circ_0001313 in discriminating rectal cancer patients from healthy controls was estimated to be of 94.0%, with a specificity of 90.0%. The positive predictive value (PPV) was of 95.9%, and the negative predictive value (NPV) was of 85.7%.

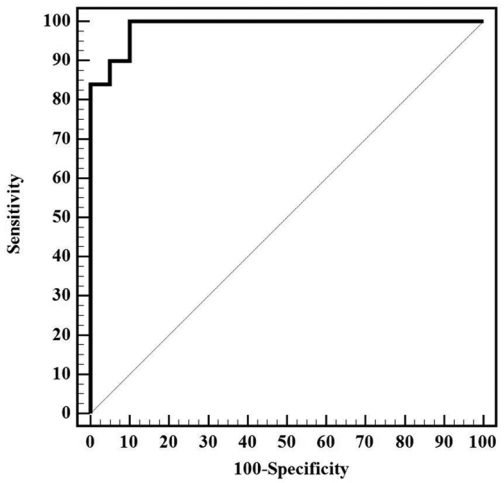

Fig. 1 Receiver operating characteristic (ROC) curve for plasma circ_0001313, to discriminate patients from controls.

Concerning the relationship between circ_0001313 and the radiological response to nCRT, there was a significant decrease in the expression level of circ_0001313 in patients in group I compared with those in groups III and IV, and a significant decrease in patients in group II compared with groups III and IV. In contrast, there was no significant difference in the plasma expression level between groups I and II or groups III and IV (►**Table 2**).

Subsequently, the groups were classified as responders (that is, groups I and II) and nonresponders (groups III and VI).[14,17] The levels of the responders were significantly lower than those of the nonresponders ($p < 0.001$).

Consequently, the analysis of the receiver operating characteristic (ROC) curve was performed to predict the response to nCRT according to the plasma expression level of circ_0001313. At the cutoff value of > 5.1337 fold change, the sensitivity of the plasma expression level in predicting those who would be nonresponders to nCRT was of 93.33%, while its specificity was of 91.43%. The PPV was of 82.4%, and the NPV, of 97.0% (►**Fig. 2**).

Correlation Studies

The correlation between sex and the level of circ_CCDC66 expression in the patients was not statistically significant ($p = 0.392$), but it was statistically significant regarding the serum levels of hemoglobin ($p = 0.609$) and CA19-9 ($p = 0.330$); moreover, we observed a positive correlation with age ($p = 0.043$).

Table 2 Comparison between the different studied groups according to the plasma expression level of circ_0001313

	Patient group ($n = 50$)					H	*p*-value
	Complete response ($n = 9$)	Partial response ($n = 26$)	Stable disease ($n = 11$)	Progressive disease ($n = 4$)	Controls ($n = 20$)		
Plasma circ_0001313							
Min– max	1.33–5.74	1.17–6.32	4.79–9.78	14.62–45.25	0.62–1.46	55.496*	< 0.001*
Median (IQR)	1.80 (1.44–3.66)	2.69 (1.71–4.11)	6.23 (5.76–8.01)	21.25 (15.2035.99)	1.06 (0.98–1.10)		
p_0	0.002*	< 0.001*	< 0.001*	< 0.001*			
Significance among groups	$p_1 = 0.814$; $p_2 = 0.011$*; $p_3 = 0.009$*; $p_4 = 0.003$*; $p_5 = 0.006$*; and $p_6 = 0.467$						

Abbreviation: H, Kruskal-Wallis test; IQR, interquartile range.
Notes: Kruskal-Wallis test: pairwise comparison between pairs of groups was performed using the Dunn Post-Hoc Test (for multiple comparisons); *p*: *p*-value for the comparison of the five studied groups; p_0: value for the comparison of the control and each of the patient groups; p_1: value for the comparison of complete and partial responses; p_2: value for the comparison of complete response and stable disease; p_3: value for the comparison of complete response and progressive disease; p_4: value for the comparison of partial response and stable disease; p_5: value for the comparison of partial response and progressive disease; and p_6: value for the comparison of stable and progressive diseases. *Statistically significant at $p \leq 0.05$.

Correlation between CEA and plasma expression of circ_CCDC66

The level of plasma expression of circ_CCDC66 was positively correlated to CEA (rs = 0.396; $p = 0.004$) considering the total sample; in each group considered separately, there was no significant correlation.

Plasma Level of Circ_0001313 and its Relationship with Pathological Response

As previously mentioned, the patients were categorized according to the pathological response using the TRG, which was conducted according to the AJCC classification as follows: TRG0–6 patients, TRG1–15 patients; TRG2–12 patients; and TRG3–17 patients. Subsequently, the patients were classified as 33 showing objective pathological responses in the form of: TRG0, TRG1, and TRG2, while 17 showed no response in the form of TRG3.[18]

Concerning the relationship of circ_0001313 to the pathological response to nCRT, there was a significant difference in the plasma expression level among the four groups ($p < 0.001$). There was a significant difference in the plasma expression level in TRG0 patients when compared with TRG3 subjects ($p = 0.003$), in TRG1 patients when compared with those who were TRG3 ($p < 0.001$), and in TRG2 subjects when compared with TRG3 patients ($p < 0.001$).

In contrast, there was no significant difference in the plasma expression level between TRG0 and TRG1, and TRG0 and TRG2 subjects. Neither was there a significant difference between TRG1 and TRG2 patients.

There was a significant difference in the plasma expression level in TRG0, TRG1 and TRG2 patients when compared with TRG3 subjects ($p < 0.001$) (►**Fig. 3**).

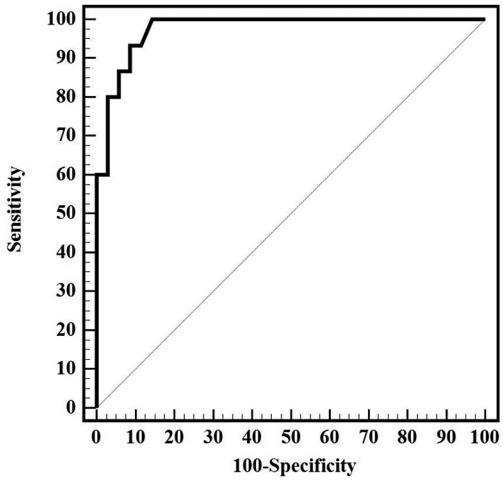

Fig. 2 Receiver operating characteristic (ROC) curve for plasma expression level of circ_0001313 to predict the response to neoadjuvant chemoradiotherapy (nCRT).

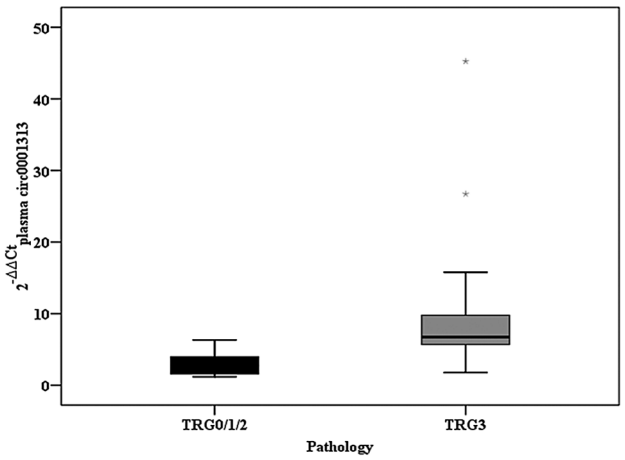

Fig. 3 Comparison between patients with pathological objective response and patients with no response according to the plasma expression level of circ_0001313.

Discussion

Rectal cancer accounts for 31% of CRC cases. It is one of the most prevalent malignancies, with a high mortality rate worldwide.[2] The combination of nCRT with surgery has transformed the therapeutic management of locally-advanced rectal cancer.[19] Because of the genome heterogeneity, and the growing field of personalized medicine, the molecular patterns of tumors were found to be different in one patient versus another, so there is a critical need for molecular predictors for individualized treatment response.[20] Circular RNAs play a role in cancer pathogenesis, with evidence[21,22] of their involvement in the resistance to cancer treatment. Nevertheless, the exact mechanism of their involvement in therapy resistance is not very clear.[23]

In the current work, we investigated the plasma expression level of circ_0001313 in 50 patients diagnosed with locally-advanced rectal cancer to determine its role as a potential non-invasive predictor of the response to nCRT; as far as we know, the present work is the first to do so.

In the present study, the plasma expression level of circ_0001313 was significantly higher in the patients relative to the controls. In agreement with the current study, Hsiao et al.[24] found that circ_CCDC66 was overexpressed in CRC cell lines, and downregulated in cell lines derived from normal epithelial cells of the colon; furthermore, tumor growth and invasion were inhibited by the knockdown of circ_CCDC66, which supports the statement that it promotes colon cancer progression. Similar findings have also been revealed by Feng et al,[25] who, in contrast to the present study, investigated the plasma expression level of three circRNAs: circ_CCDC66, circ_ABCC1, and circ_STIL, and they found that their levels were significantly downregulated in CRC patients when compared with healthy controls. They[25] also found that a combination of these 3 circRNAs with CEA and CA19-9 could improve the ability to diagnose CRC. Finally, they[25] suggested that circ_CCDC66 could be a potential non-invasive predictive biomarker for CRC that decreases in the plasma of CRC patients in comparison with healthy controls. We can speculate that the difference may be due to heterogeneity and specificity of secretory mechanisms. The molecular mechanisms that control the secretion process of plasma circRNAs are not yet fully understood. Moreover, the expression of circRNAs exhibits differences between blood and tissues, which is also related to secretory mechanisms. Another explanation is that the origin of circRNAs is variable; they may originate from tumor cells themselves or other cells in the tumor microenvironment.[26]

Regarding circ_0001313 expression in different cancers, studies have shown that circ_0001313 is often overexpressed in various cancers and acts as an oncogene, promoting tumor invasion. For instance, in nonsmall cell lung cancer (NSCLC), both tissues and cell lines showed upregulation of circ_0001313, which was linked to decreased levels of microRNA (miRNA) 452 (miR-452) expression. Moreover, the suppression of circ_0001313, in turn, suppresses NSCLC cell proliferation and invasion.[27] It has been suggested[27] that circ_0001313 may act as an oncogenic rather than a tumor suppressor. A possible explanation for this can be found in a study on gastric cancer by Yang et al.,[28] who reported that circ_CCDC66 was upregulated in gastric cancer patients and implicated in the proliferation and invasion of gastric and colon cancer by acting as an miRNA "sponge" to sequester miRNAs. Furthermore, in the present study, the diagnostic value of circ_CCDC66 was tested, and the ROC curve analyses showed that circ_CCDC66 could discriminate rectal cancer patients from the controls with a sensitivity of 94.0% and specificity of 90%.

The patients were further classified according to their response to nCRT: 18% presented complete response; 52%, partial response; and 30%, no response (22% showing stable disease and 8%, progressive disease).There were statistically significant differences in circ_0001313 expression level among the four studied groups (►**Table 2**). It was significantly overexpressed in patients with resistance to nCRT (stable and progressive diseases) in comparison with responders (complete pathological and partial responses) (►**Table 3**). There was statistically significant relationship between the plasma expression level of circ_0001313 and response to nCRT (using the AJCC TRG system).

Regarding the response to treatment and development of therapy resistance, Wang et al.[29] explored the functions of circ_0001313 in regulating the radiosensitivity in colon cancer. They found that circ_0001313 was overexpressed and miR-338–3p was downregulated in the colon cancer tissues compared with normal tissues. The researchers[29] also detected the

Table 3 Comparison between the groups of responders and nonresponders according to the plasma expression level of circ_0001313

	Patient group (n = 50)		*p*-value
	Responder (n = 35)	Non-responder (n = 15)	
$2^{-\Delta\Delta Ct}$ plasma circ_0001313			
Min–max	1.17–6.32	4.79–45.25	< 0.001*
Median (IQR)	2.62 (1.64–3.85)	7.21 (5.84–12.20)	

Abbreviations: H, Kruskal-Wallis test; IQR, interquartile range.
Notes: Pairwise comparison between groups was performed using the Dunn Post-Hoc Test (for multiple comparisons). * Statistically significant at $p \leq 0.05$.

expression of circ_0001313 in radioresistant and radiosensitive colon cancer tissues: they found that, in radioresistant colon cancer tissues, circ_0001313 was significantly overexpressed, while miR-338–3p expression was decreased compared with the radiosensitive tissues. These results[29] suggest that circ_0001313 may be involved in radioresistance development in CRC by sponging miR-338–3p. Lin et al.[30] demonstrated that CRC cells with oxaliplatin resistance had elevated expression levels of circ_CCDC66. Knockdown of circ_CCDC66 through oligonucleotides of small interfering RNA (siRNA) in resistant cells treated with oxaliplatin decreased the survival of resistant malignant cells and increased the apoptotic effects of oxaliplatin. The authors[30] suggested that circ_CCDC66 could be implicated in the survival of resistant cells by opposing oxaliplatin-induced cell death; this action might occur through modulation of genes involved in cell proliferation and survival. It has been found that the suppression of circ_CCDC66 hindered the proliferation and invasive ability of tumors. Moreover, circ_CCDC66 was found to be implicated in the development of cisplatin resistance in GC patients.[28] An increased expression level of circ_0001313 was found in cisplatin-resistant patients, and a ROC curve analysis was performed to evaluate its predictive performance; the area under the curve was of 81.5%, supporting the statement that circ_CCDC66 may be a valid predictive biomarker for cisplatin resistance in GC patients.[12] Thus, suppressing the expression of circ_CCDC66 in cancers could prevent tumor invasion and progression and hold therapeutic potential.

Conclusion

We found that the expression of circ_CCDC66 is increased in rectal cancer patients. This suggests that circ_CCDC66 may have an oncogenic function and could be used as a diagnostic marker for CRC. Rectal cancer patients with higher circ_CCDC66 expression levels were found to be more resistant to nCRT. Based on these results, circ_0001313 could be a promising noninvasive stable biomarker to predict the pathological and radiological responses to nCRT in patients with locally-advanced rectal cancer.

Limitations and Recommendations

Since the present was a single-center study with a relatively small sample size, and limited geographical distribution of participants, these findings need to be confirmed with larger sample sizes and across multiple centers. Further investigations with more varied sample sources and measuring the expression level of other circRNAs, along with associated RNAs and proteins, is recommended as well. This will result in a better understanding of their functional mechanisms and could open new therapeutic perspectives for colon cancer patients.

Authors' Contributions

All authors contributed equally to; data collection, implementation of the experiment, analysis and interpretation of results. The first draft of the manuscript was written and edited by Hend M Batea, Doaa A Abdelmonsif, Gehan M Khedr, and Ahmed Moaz. Safaa H Moy El-dine and Eman M Kamha reviewed the manuscript and approved the final version.

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Conflict of Interests

The authors have no conflicts of interests to declare.

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