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ceRNA Networks: The Backbone Role in Neoadjuvant Chemoradiotherapy Resistance/Sensitivity of Locally Advanced Rectal Cancer

Lin He, PhD[1,2,*], Hao Chang, MD[1,*], Yuhong Qi, MD[1], Bing Zhang, MM[1], and Qiuju Shao, MD[1]

Abstract

Approximately 40% of rectal cancers during initial diagnosis are identified as locally advanced rectal cancers (LARCs), for which the standardized treatment scenario is total mesorectal excision following neoadjuvant chemoradiotherapy (nCRT). nCRT can lead to discernible reductions in local relapse rate and distant metastasis rate in LARC patients, in whom previously inoperable tumors may potentially be surgically removed. However, only 4% to 20% cases can attain pathological complete response, and the remaining patients who are unresponsive to nCRT have to suffer from the side effects plus toxicities and may encounter poor survival outcomes due to the late surgical intervention. As such, employing potential biomarkers to differentiate responders from nonresponders before nCRT implementation appears to be the overarching goal. Well-defined competing endogenous RNA (ceRNA) networks include long noncoding RNA (lncRNA)-microRNA (miRNA)-mRNA and circRNA-miRNA-mRNA networks. As ceRNAs, lncRNAs, and circRNAs sponge miRNAs to indirectly suppress miRNAs downstream of oncogenic mRNAs or tumor-suppressive mRNAs. The abnormal expression of mRNAs regulates the nCRT-induced DNA damage repair process through pluralistic carcinogenic signaling pathways, thereby bringing about alterations in the nCRT resistance/sensitivity of tumors. Moreover, many molecular mechanisms relevant to cell proliferation, metastasis, or apoptosis of cancers (eg, epithelial-mesenchymal transition and caspase-9-caspase-3 pathway) are influenced by ceRNA networks. Herein, we reviewed a large group of abnormally expressed mRNAs and noncoding RNAs that are associated with nCRT resistance/sensitivity in LARC patients and ultimately pinpointed the backbone role of ceRNA networks in the molecular mechanisms of nCRT resistance/sensitivity.

Keywords

neoadjuvant chemoradiotherapy, DNA damage repair, locally advanced rectal cancer, competing endogenous RNAs

Abbreviations

LARC, locally advanced rectal cancer; nCRT, neoadjuvant chemoradiotherapy; pCR, pathological complete response; OS, overall survival; TRG, tumor regression grade; ncRNAs, non-coding RNAs; NHEJ, non-homologous end joining; HR, homologous recombination; ERCC, excision repair cross-complementing; CHD4, chromodomain helicase DNA-binding protein 4; NER, nucleotide excision repair; TYMS, thymidylate synthase; EGFR, epidermal growth factor receptor; DNA-PK, DNA-dependent protein kinase; CoA, coenzyme A; *COASY*, CoA synthase; *AEG-1*, astrocyte elevated gene-1; *CCR6*, C-C motif chemokine receptor 6; *COX-2*, cyclooxygenase-2; PGE2, prostaglandin E2; CCND1, cyclin D1; *NDRG1*, *N-myc* downstream-regulated gene 1; EMT, epithelial-mesenchymal transition; *ZEB1*, zinc finger E-box binding homeobox 1; CSCs, cancer stem cells; *RBBP6*, retinoblastoma binding protein 6; sncRNAs, short ncRNAs; lncRNAs, long ncRNAs; miRNAs, microRNAs; UTR, untranslated region; ceRNAs, competing endogenous RNAs; MREs, miRNA recognition elements.

[1] Department of Radiotherapy, Tangdu Hospital, Air Force Military Medical University, Xi'an, Shaanxi Province, China
[2] Cancer Centre, Faculty of Health Sciences, University of Macau, Macau, SAR, China

[*] These authors are contributed equally to this work.

Corresponding Author:
Qiuju Shao, Department of Radiotherapy, Tangdu Hospital, Air Force Military Medical University, No. 1 Xinsi Road, Baqiao District, Xi'an, 710032 Shanxi Province, China.
Email: shaoqjfmmu@163.com

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Introduction

Colorectal cancer is the third most common carcinoma and the second most common cause of cancer-related death in the world.[1] Approximately 30% of colorectal cancers are rectal cancers, of which 40% are definitively identified as locally advanced rectal cancer (LARC) during initial diagnosis.[2,3] It is significant that rectal cancer has experienced a steadily reduced incidence in developed countries but has increased to a rate of 4.2% per year in mainland China, establishing it as the fifth leading cause of cancer-related mortality in this country.[4,5] Currently, the standardized treatment paradigm for LARC patients is total mesorectal excision after neoadjuvant chemoradiotherapy (nCRT), which results in a resounding shrinkage of the local relapse rate and an increased pathological complete response (pCR) rate.[6,7] In parallel, nCRT may provide an opportunity for radical surgery on previously inoperable tumors. Nevertheless, only 4 to 20% of postnCRT LARC patients can achieve pCR.[8] The remaining patients who are inert to nCRT not only suffer from side effects and toxicities but also have a high risk of poor prognosis due to late surgical interventions. Therefore, it is crucial that a distinction is made between the nCRT-sensitive cohort and the nCRT-resistant cohort by predicting which patients may benefit from nCRT. In this case, the sensitive cohort will receive nCRT directly, and the resistant cohort will be spared from nCRT and triaged to local resection or other mild therapeutic regimens.

nCRT resistance gives rise to tumor recurrence and metastasis and is a direct reason for polytherapeutic failure and the shortened overall survival (OS) of LARC patients; thus, there is an urgent need to overcome nCRT resistance and ameliorate nCRT sensitivity. After nCRT, tumor regression grade (TRG) is an independent factor influencing the long-term survival of LARC patients.[9] Mandard *et al*[10] in 1994 defined TRG, which was divided into 5 histologic grades: TRG1 (complete regression), no histologically recognizable residual tumor cells and fibrosis permeating all layers of the esophageal wall; TRG2, rare residual tumor cells scattered in the fibrosis; TRG3, presence of an increased number of residual cancer cells but still with a lower proportion than fibrosis; TRG4, residual cancer cells overgrowing fibrosis; and TRG5 (complete nonregression), absence of tumor regressions. Generally, postnCRT patients with TRG1-2 are considered to be the sensitive cohort, while those with TRG3-5 belong to the resistant cohort.[11]

The growing appreciation of the molecular mechanisms in nCRT resistance/sensitivity and the investigation of potential biomarkers for differentiating sensitive cohorts from resistant cohorts have evolved through many clinical studies using the above definitive classifications. Herein, we reviewed relevant biomarkers from mRNA and noncoding RNA (ncRNA) domains within the present molecular mechanisms of nCRT resistance/sensitivity in LARC patients. In addition, we summarized or recalculated the prognostic performance of many potential biomarkers in distinguishing sensitive cohorts and resistant cohorts in terms of the original data from the included studies.

nCRT Resistance/Sensitivity-Related mRNAs

DNA Damage Repair Pathway

The most classical mechanism for tumorous CRT resistance is DNA damage repair (DDR),[12] which predominantly includes 2 mainstays, the nonhomologous end joining (NHEJ) pathway and the homologous recombination (HR) pathway. The DNA repair proteins Ku70 and Ku80 participate in the DDR process by the NHEJ pathway, which is crucial for the G1 to S phase. Concomitantly, the DNA repair proteins RAD23B and RAD51 are involved in the HR pathway, which plays a key role in the S to G2 phase and first needs to form a heterodimer with a variety of excision repair cross-complementing (ERCC) proteins (eg, ERCC1, ERCC2, XRCC2, XRCC3, and BRCA2) (Figure 1).

Dysregulated mRNA-Induced nCRT Resistance/Sensitivity via the DNA Damage Repair Pathway

Chromodomain helicase DNA-binding protein 4 (CHD4), an important subunit of the nucleosome-remodeling and histone deacetylation chromatin-remodeling complex, is involved in the DDR process and maintains genomic integrity and stability via the HR pathway.[13] In rectal cancer cells, *CHD4* mRNA, in cooperation with DNA methyltransferases, can silence many tumor-suppressive genes (eg, *MLH1*, *SFRP1*, *SFRP2*, *SFRP4*, *TIMP2*, and *TIMP3*), thus driving the Wnt/β-catenin signaling pathway.[14] Findings indicated that upregulated *CHD4* mRNA performed well in the prognosis of nCRT resistance in LARC patients (accuracy = 60%; Table 1), and *CHD4* mRNA knockdown enhanced nCRT sensitivity.[19] The nucleotide excision repair (NER) pathway shows a major impact on nCRT resistance, which requires the participation of multiple ERCC proteins (eg, ERCC1 and ERCC2).[32-34] Overexpressed *ERCC1* mRNA in LARC patients can elicit nCRT resistance by accelerating the NER pathway and results in a poor prognosis, with reliable predictive performance of nCRT resistance (accuracy = 73%; Table 1).[22]

Thymidylate synthase (TYMS) is a downstream targeted molecule of 5-fluorouracil (5-FU) chemotherapy and is essential for DNA synthesis.[35] Upregulation of *TYMS* mRNA maps to 5-FU resistance in LARC patients.[36] Furthermore, positive expression of *TYMS* mRNA in circulating tumor cells is an effective biomarker to predict nCRT resistance (accuracy =

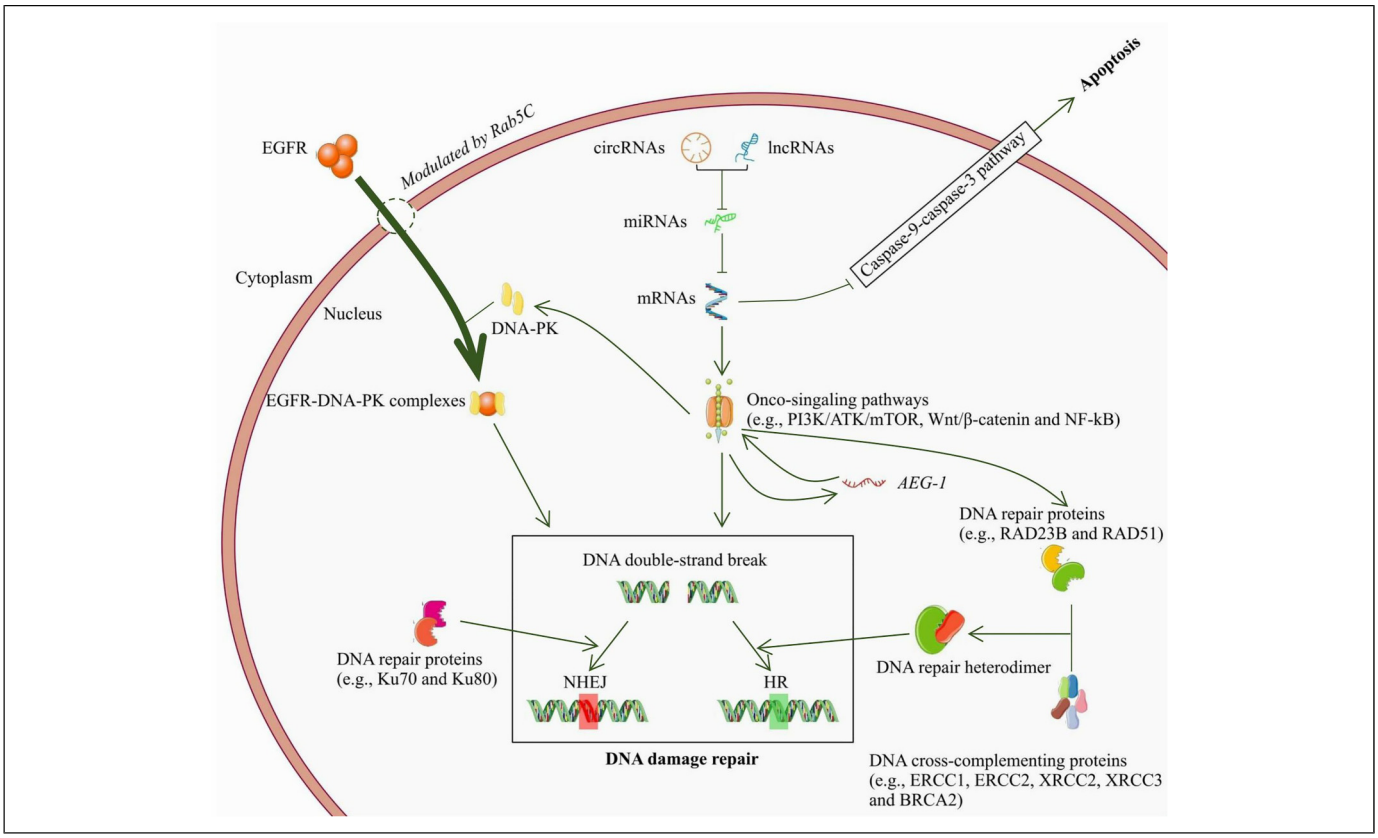

Figure 1. The backbone role of ceRNA networks in neoadjuvant chemoradiotherapy resistance/sensitivity of rectal cancers.
ceRNAs sponge their downstream targeted miRNAs that suppress the expression level of miRNA-targeted mRNAs. mRNAs regulate the DNA damage repair (DDR) process through several carcinogenic signaling pathways. The DDR is the most classic mechanism for tumorous chemoradiotherapy (CRT) resistance and includes the nonhomologous end joining (NHEJ) pathway and homologous recombination (HR) pathway. The DNA repair proteins Ku70 and Ku80 are involved in the NHEJ pathway, whereas the DNA repair proteins RAD23B and RAD51 require excision repair cross-complementing proteins to form a DNA repair heterodimer that participates in the HR pathway. Oncogenes activate astrocytic elevated gene-1 (*AEG-1*) via the PI3 K/AKT/mTOR pathway, and *AEG-1*, in turn, drives multiple tumorigenic signaling pathways, including the PI3 K/AKT/mTOR, Wnt/β-catenin, and NF-kB pathways. Moreover, oncogenes suppress caspase-9-caspase-3-mediated apoptosis, thereby enhancing CRT resistance. Activated epidermal growth factor receptor (EGFR) internalizes into the nucleus, followed by the formation of a new complex with DNA-dependent protein kinase (DNA-PK). The newly formed complex is available to accelerate the DDR process. The internalization of EGFR is modulated by *Rad5C*. Some tumorigenic signaling molecules (eg, Akt) can maintain the integrity and stability of DNA-PK and RAD51, which leads to the increased CRT resistance of rectal tumors.

83%; Table 1) and is not detected in any responders,[21] suggesting that *TYMS*-negative patients are sensitive to nCRT. RAD18 is an E3 ubiquitin-linked enzyme that maintains the integrity and stability of the genome through several DNA repair pathways, by which overexpressed *RAD18* mRNA leads to CRT resistance in multiple human cancers.[8,37] Increased *RAD18* mRNA expression concurrently inhibits caspase-9-caspase-3-mediated apoptosis in rectal cancers, which further reinforces nCRT resistance (Figure 1).[8] A previous clinical study corroborated the good prognostic performance of overexpressed *RAD18* mRNA in identifying nCRT resistance in LARC patients (accuracy = 65%; Table 1).[8]

Epidermal growth factor receptor (EGFR) indirectly engages in the regulation of the DDR process. Specifically, EGFR can be activated by irradiation and then internalized into the nucleus to form a complex with DNA-dependent protein kinase (DNA-PK), which is associated with the promoted DDR process.[38,39] *Rab5C* modulates the internalization process of EGFR and elevates the expression of Ku70 and Ku80,[40] thus enhancing CRT resistance in rectal cancers (Figure 1). In light of these findings, a clinical study found that *EGFR* mRNA-negative results combined with *VEGF* mRNA-positive results performed well in differentiating the nCRT resistant cohort from the nCRT sensitive cohort (accuracy = 65%; Table 1).[23]

The PI3 K/AKT/mTOR signaling pathway changes the CRT resistance of cancer cells via direct regulation of the DDR process.[27] Coenzyme A (CoA) and its derivatives take part in multiple pathways of cell metabolism, including pyruvate oxidation, fatty acid synthesis, cell cycle processes, and cell death.[41] Human rectal cancer enriches CoA synthase (*COASY*), which causes an inferior response to CRT by activating the PI3 K/AKT/mTOR signaling pathway,[17] while the overexpression of *COASY* mRNA has value in predicting nCRT resistance in LARC patients (accuracy = 67%; Table 1). The oncogene *Ha-Ras* uses the PI3 K/AKT/mTOR signaling pathway to activate astrocyte elevated gene-1 (*AEG-1*)

Table 1. Prognostic Performance of Overexpressed Biomarkers in Predicting nCRT Resistance and nCRT Sensitivity of Locally Advanced Rectal Cancer.

Biomarkers	Biological sample	Sensitive cohort (N)	Resistant cohort (N)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Ref.
nCRT resistance									
Nucleic β-catenin	Primary tumor	118	18	65	88	79	48	83	15
CCR6	Primary tumor	40	55	76	63	74	66	71	16
COASY	Primary tumor	13	20	65	69	76	56	67	17
CD133	Primary tumor	30	46	76	51	78	65	72	18
CHD4	Primary tumor	135	37	70	58	31	88	60	19
Pim-3	Primary tumor	85	90	87	39	60	73	63	20
TYMS	blood	18	12	83	83	88	77	83	21
RAD18	Primary tumor	24	27	85	42	62	71	65	8
ERCC1	Primary tumor	57	29	83	68	57	89	73	22
EGFR-/VEGF+	Primary tumor	27	61	52	93	94	46	65	23
miR-21	Primary tumor	10	60	87	60	93	43	83	24
miR-31	Primary tumor	55	23	61	76	52	82	72	25
miR-487a-3p	Primary tumor	67	20	78	60	37	91	64	26
nCRT sensitivity									
miR-21-5p	Primary tumor	7	20	100	85	70	100	89	27
miR-1246	Primary tumor	7	20	86	65	46	93	70	27
miR-1290-3p	Primary tumor	7	20	71	75	50	88	74	27
miR-205-5p	Primary tumor	7	20	86	55	40	92	63	27
miR-519b-3p	Primary tumor	21	34	100	81	87	100	92	28
miR-200c	Primary tumor	12	30	92	73	58	96	79	29
miR-223	Primary tumor	12	9	100	78	86	100	90	30
KLF7-1/ MAB21L2-1/ LINC00324	Primary tumor	18	12	91	94	94	85	90	31

Abbreviations: PPV, positive predictive value; NPV, negative predictive value.

mRNA, which in turn prompts the activation of multiple carcinogenic signaling pathways, eg, PI3 K/AKT/mTOR, Wnt/β-catenin, and NF-kB (Figure 1).[42-46] Overexpressed *AEG-1* mRNA is an independent risk factor for disease-free survival and distant metastasis-free survival of postnCRT LARC patients,[47] meaning that it can be used as a potential biomarker for the prognosis of nCRT resistance.

There is unequivocal evidence that C-C motif chemokine receptor 6 (*CCR6*) mRNA facilitates the oncogenesis, progression, and metastasis of rectal cancer.[48,49] Additionally, upregulated *CCR6* mRNA maintains the integrity and stability of DDR direct participants (eg, DNA-PK and RAD51) by activating its downstream signaling molecules, that is, Akt and ERK, thus triggering nCRT resistance and attenuating nCRT efficacy in LARC patients (Figure 1).[16] Table 1 notes that overexpression of *CCR6* mRNA was a reliable biomarker in the prognosis of nCRT resistance in LARC patients (accuracy = 71%).

Cyclooxygenase-2 (*COX-2*), a well-known inflammatory reaction factor, demonstrates critical functions in tumorigenesis, including progression, metastasis, and angiogenesis.[50-53] Prostaglandin E2 (PGE2)-induced transformations extend the G1 phase by strengthening the expression of cyclin D1 (CCND1) and establishing the antiapoptotic function of PGE2.[54] *COX-2* fosters PEG2 production; thus, the inhibition of apoptosis occurs.[55] *COX-2* mRNA overexpression is positively associated with the poor prognosis of post-nCRT LARC patients,[56] and accordingly, there is a postulation that the administration of *COX2* inhibitors prior to nCRT may reduce the distant metastasis rate and prolong OS.

N-myc downstream-regulated gene 1 (*NDRG1*) maintains the function of E-cadherin and β-catenin, which are both involved in cell adhesion and localization and are thought to be potential tumor metastasis suppressors.[57] Interestingly, *NDRG1* mRNA overexpression gives rise to elevated expression levels of CRT resistance-related proteins, eg, MDR, LRP-1, and MRP-1.[58] Gene silencing of *NDRG1* mRNA makes rectal cancer cells sensitive to CRT by creating more DNA double-strand breakages.[59] Epithelial-mesenchymal transition (EMT) constitutes the molecular mechanism of tumorous CRT resistance, in which nucleic β-catenin accumulates, E-cadherin is reduced, miR-200c is underexpressed, and tumor budding begins to advance.[29,60] Nucleic β-catenin was significantly increased in the nCRT-resistant cohort of LARC patients relative to the nCRT-sensitive cohort of those patients (57.6% vs 16.7%, $P < .001$), and utilizing it as a single biomarker to predict nCRT resistance showed an effective performance (accuracy = 83%; Table 1).[15] *OCT4* is a key transcription factor in embryonic stem cells that can render LARC patients resistant to nCRT by accelerating the EMT process. In detail, *OCT4* mRNA upregulates the expression of an EMT-related transcription factor, zinc finger E-box binding homeobox 1 (*ZEB1*), and gene silencing of *ZEB1* in turn can reverse *OCT4* mRNA-induced nCRT resistance.[61] Collectively, *OCT4* mRNA is *ZEB1*-dependent in its elevation of nCRT resistance in LARC patients.

Cancer stem cells (CSCs) exhibit an inherent antiapoptotic nature and robust CRT resistance due to their intrinsic DDR capability.[62] *CD133* mRNA is a specific biomarker for multiple phenotypes of CSCs, and its overexpression implies the probable presence of nCRT resistance in LARC patients (accuracy = 72%; Table 1).[18] Since retinoblastoma binding protein 6 (*RBBP6*) demonstrates the capability to bind with the tumor suppressors p53 and Rb, it may be involved in the cell cycle, proliferation, apoptosis, and CRT resistance of tumor cells.[63-65] Overexpressed *RBBP6* mRNA arrests the G2 to M phase and regulates the apoptosis pathways, which leads to CRT resistance of rectal cancer cells. In contrast, inhibiting cellular *RBBP6* expression predisposes rectal tumors to increased CRT sensitivity.[66] *Pim-3* is a member of the *Pim* family that is recognized as an anti-apoptotic oncogene and causes tumor growth by synergistically working with the proto-oncogene *Myc*.[20] Studies have shown that the expression of *Pim-3* in colorectal cancer tissues was significantly increased compared to that in normal colon tissues (32.6% vs 0.02%; $P < .001$).[67] The pCR rate in LARC patients with overexpressed *Pim-3* mRNA was significantly lower than that in patients with underexpressed *Pim-3* mRNA ($P = 0.001$),[20] indicating good prognostic performance of the overexpressed *Pim-3* mRNA in differentiating the nCRT-resistant cohort from the nCRT-sensitive cohort (accuracy = 63%; Table 1).

Direct Evidence of Dysregulated mRNA-Induced nCRT Resistance/Sensitivity

In addition to the aforementioned mRNAs involved in the molecular mechanisms of nCRT resistance, a large body of studies have identified a direct correlation between many upregulated mRNAs[68-82] or other downregulated mRNAs[72,83,84] and the nCRT resistance of LARC patients (Table 2), indicating that they can be used as potential biomarkers to predict postnCRT prognosis. For example, overexpression of *KRAS*, *PDPK*, *PPP2R5C*, and *YES1* mRNAs and underexpression of *PPP2R1B* mRNA are responsible for the poor prognosis of postnCRT LARC patients via activation of the PI3 K/AKT/mTOR signaling pathway.[72]

nCRT Resistance/Sensitivity-Related miRNAs

Dysregulated miRNA-Induced nCRT Resistance/Sensitivity Against Downstream Targeted mRNAs

Several studies have only evaluated the association between dysregulated miRNAs and the abnormal expression of downstream targeted mRNAs and CRT resistance in rectal cancers. As previously reviewed, *XRCC3* mRNA encodes one of the kernel proteins in the HR pathway; the expression level of *XRCC3* mRNA in rectal cancers can be increased by the downregulation of miR-185,[89] and CRT resistance is thereby enhanced. Rectal cancers treated with CRT overexpress miR-95, which suppresses its downstream targeted sphingolipid phosphatase *SGPP1* mRNA, resulting in weakened tumor necrosis and reinforced cell proliferation.[90] These results highlight the core role of miR-95 in nCRT resistance in LARC patients.

In contrast, some studies have revealed that overexpressed miRNAs increase the CRT sensitivity of rectal tumors by regulating their downstream targeted mRNAs. miR-205 suppresses its downstream targets *ZEB1* mRNA and *Ubc13* mRNA to blockade the DDR process.[91] miR-130a hampers the DDR process by directly targeting *SOX4* mRNA and dramatically reverses the EMT phenotype of rectal cancer cells.[92] Consequently, there is a likelihood that miR-205/miR-130a may function as a CRT sensitizer in the treatment of rectal cancers and be utilized as a potential therapeutic target to improve post-CRT prognosis. miR-451a is expressed at significantly higher levels in the nCRT-sensitive cohort of LARC patients than in the nCRT-resistant cohort of those patients, whereas its downstream targets *CAB39* mRNA and *EMSY* mRNA tend to be expressed at lower levels,[93] suggesting that miR-451a-induced nCRT sensitivity is based on the suppression of *CAB39* mRNA and *EMSY* mRNA. The overexpression of *ARID4B* mRNA is reported to be positively correlated with

Table 2. Direct Evidence of Abnormal RNA Expression-Induced nCRT Resistance in Locally Advanced Rectal Cancer.

RNAs	Biological sample	Evaluation technique	Expression status	*P* value*	Ref.
mRNAs					
HER2	Primary tumor	IHC	↑	.026	68
FGF8	Primary tumor	IHC	↑	.003	69
CLCA1	Primary tumor	IHC	↑	.042	70
IGF-1R	Primary tumor	IHC	↑	<.001	71
KRAS	Primary tumor	IHC	↑	<.01	72
PDPK1	Primary tumor	IHC	↑	<.01	72
PPP2R5C	Primary tumor	IHC	↑	<.01	72
YES1	Primary tumor	IHC	↑	<.01	72
BRAF	Primary tumor	IHC	↑	.012	73
SMAD4	Primary tumor	IHC	↑	.02	73
PITPNC1	Primary tumor	IHC	↑	<.05	74
CIP2A	Primary tumor	IHC	↑	.006	75
SATB1	Primary tumor	IHC	↑	<.001	76
AC	Primary tumor	ELISA	↑	<.00001	77
YKL-40	Primary tumor	IHC	↑	<.01	78
c-Met	Primary tumor	IHC	↑	.006	78
Rsf-1	Primary tumor	IHC	↑	.028	79
LKB1/LGR5	Primary tumor	IHC	↑	<.05	80
GLUT1	Primary tumor	IHC	↑	<.0001	81
ANXA1	Primary tumor	IHC	↑	.009	82
PPP2R1B	Primary tumor	IHC	↓	<.01	72
PSMB8	Primary tumor	RT-PCR	↓	.001	83
SLC39A7	Primary tumor	RT-PCR	↓	.012	83
53BP1	Primary tumor	IHC	↓	<.05	84
miRNAs					
miR-215	Primary tumor	miRNA assay	↑	.04	11
miR-190b	Primary tumor	miRNA assay	↑	.029	11
miR-29b-2	Primary tumor	miRNA assay	↑	.0375	11
miR-31	Primary tumor	miRNA assay	↑	.018	25
miR-487a-3p	Primary tumor	RT-PCR	↑	.0006	26
miR-125b	Primary tumor	RT-PCR	↑	.023	85
miR-137	Primary tumor	RT-PCR	↑	.002	85
miR-345	Blood	qRT-PCR	↑	.046	86
miR-374a-5p	Blood	miRNA assay	↑	<.0001	87
miR-224	Tumor cells	RT-PCR	↑	<.0001	88
let-7e	Primary tumor	miRNA assay	↓	.0075	11
miR-196b	Primary tumor	miRNA assay	↓	.043	11
miR-450a	Primary tumor	miRNA assay	↓	.0104	11
miR-450b-5p	Primary tumor	miRNA assay	↓	.0003	11
miR-99a	Primary tumor	miRNA assay	↓	.0163	11
miR-223	Primary tumor	RT-PCR	↓	<.01	30
miR-342-5p	Blood	miRNA assay	↓	.044	87
miR-519d-3p	Blood	miRNA assay	↓	.014	87
miR-320a	Tumor cells	RT-PCR	↓	<.0001	88
miR-132	Tumor cells	RT-PCR	↓	<.0001	88
let-7g	Tumor cells	RT-PCR	↓	<.0001	88

Abbreviations: IHC, immunohistochemistry; ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcription-polymerase chain reaction; qRT-PCR, quantitative RT-PCR.
*$P < .05$ means statistical significance.

tumorigenesis, invasion, and metastasis of breast cancers.[94] miR-519b-3p directly bonds to the 3′-UTR of *ARID4B* mRNA, giving rise to *ARID4B* mRNA underexpression that improves CRT sensitivity;[28] thus, the upregulation of miR-519b-3p has an excellent predictive performance in identifying nCRT sensitivity in LARC patients (accuracy = 92%; Table 1). An effective prognostic performance in defining the nCRT sensitivity of LARC patients is also exhibited by miR-21-5p (accuracy = 89%; Table 1), which increases tumorous nCRT sensitivity predominantly by targeting *ASTB1* mRNA.[27] Overexpressed miR-194 indirectly curbs the Wnt/β-catenin signaling pathway by suppressing the expression of

Table 3. Techniques for Identifying lncRNAs and circRNAs.

Tools	Functions	References
lncRNAs		
RNA-Seq	Characterizes and annotates lncRNAs and provides the abundance and exonic structure of the RNAs, allowing for better understanding of alternative splicing.	107
CaptureSeq	Enriches transcripts of interest by hybridizing them to magnetic bead-linked oligonucleotides, allowing for targeted purification, multiplexed library preparation and RNA sequencing at a high depth.	108
RIP-Seq	Captures transcriptome and identifies RNA-protein interaction.	109
ChIRP-Seq	Illuminates the intersection of RNA and chroatin with newfound precision genome wide.	110
RNA-FISH	Quantifies the single RNA molecules and visualizes the location of lncRNAs with cells.	111
RNA-3C	Detects the lncRNA-DNA interaction.	106
Structure-Seq	Infers the secondary structure of RNA.	112
CRISPR/cas9	Engineers knock-out or knock-in of lncRNAs	113
ASO	Perturbs the interaction of lncRNAs with proteins, DNAs or other RNAs.	114
circRNAs		
RPD	Investigates putative protein-binding partners by using probes for known circRNAs.	115,116
RIP	Analyzes circRNA-protein interactions.	115,116
FISH/ISH	Detects circRNA-protein binding by using DNA oligo probes, and fluorescently labeled antibodies, allowing for the determination of binding sites.	115,116
RPA	Detects RNA, and RNA fragments and maps circRNA-protein interactions.	117
EMSA	Studies DNA-protein and RNA-protein interactions.	118

Abbreviations: RNA-Seq, RNA sequencing; CaptureSeq, capture sequencing; RIP, RNA immunoprecipitation; ChIRP, chromatin isolation by RNA purification; FISH/ISH, fluorescence in situ hybridization; RNA-3C, RNA-chromosome conformation capture; ASO, antisense oligonucleotides; RPD, RNA pull-down assay; RPA, RNase protection assay; EMSA, electrophoretic mobility shift assay.

its downstream target *TRAF6* mRNA, leading to elevated nCRT sensitivity in LARC patients.[95]

Although the abovementioned articles report that many miRNAs targeting their downstream targeted mRNAs are closely related to the varied nCRT resistance of LARC patients, there is less understanding of the upstream targets of these miRNAs, that is, lncRNAs and circRNAs. This presents challenges in biologically constructing nCRT resistance-related ceRNA networks in LARC patients and increases the difficulty of developing inhibitors against potential biomarkers.

Direct Evidence of Dysregulated miRNA-Induced nCRT Resistance/Sensitivity

Several studies have only investigated the expression difference of miRNAs between the nCRT-resistant cohort and the nCRT-sensitive cohort. The overexpression of miR-21 (accuracy = 83%), miR-31 (accuracy = 72%), and miR-487a-3p (accuracy = 64%) showed good to effective performance in predicting nCRT resistance in LARC patients,[24-26] while the overexpression of miR-1246 (accuracy = 70%), miR-1290-3p (accuracy=74%), miR-205-5p (accuracy = 63%), miR-200c (accuracy = 79%), and miR-223 (accuracy = 90%) revealed good to excellent predictive performance in ascertaining the nCRT sensitivity of LARC patients (Table 1).[27,29,30] Compared to the nCRT-sensitive cohort, many phenotypes of miRNAs are upregulated[11,25,26,85-88] or downregulated[11,30,87,88] in tumors or biological fluids (ie, blood, saliva, and urine) of the nCRT-resistant cohort (Table 2), and these dysregulated miRNAs in turn could be used to discern nCRT sensitivity or nCRT resistance.

nCRT Resistance/Sensitivity-Related ceRNAs

Competing Endogenous RNAs Networks

The classifications of ncRNAs include short ncRNAs (sncRNAs), long ncRNAs (lncRNAs), and circRNAs; sncRNAs are further divided into Piwi-interacting RNAs, small interfering RNAs, tRNAs, rRNAs, snoRNAs, and microRNAs (miRNAs).[96,97] miRNAs are highly conserved, with lengths of 18 to 25 nucleotides, and regulate gene expression at the posttranscriptional level by degrading and/or translationally repressing their downstream targeted mRNAs.[98] A recent study suggested that miRNAs mediate the expression level of many oncogenes, antioncogenes, and CRT resistance-related genes.[99] lncRNAs, endogenous ncRNAs with a length over 200 nucleotides, frequently do not encode proteins and have the following major biological contributions: transcriptional regulation, encoding modulation, and organization of nuclear domains.[100,101] Studies have revealed that the dysregulation of lncRNAs is associated with carcinogenesis and CRT resistance.[102] circRNAs are endogenous ncRNAs lacking 5′- and 3′-untranslated regions (UTRs) which are produced by backsplicing of precursor mRNAs.[103] They have a circular structure, evolve conservatively, and are highly stabilized, thereby showing inherent resistance to RNase but can exhibit abnormal expression when they are mediated in cancer progression.

By August 2011, Salmena *et al*[104] presented a "competing endogenous RNAs (ceRNAs)" hypothesis that communication

across all types of RNA transcripts uses the letters of a new language, called miRNA recognition elements (MREs). This communicated information will ultimately be "heard" and translated by using an increasing body of updated experimental techniques. lncRNAs and circRNAs are affiliated with ceRNAs, mechanically called miRNA sponges that indirectly regulate miRNAs downstream of targeted mRNAs by sponging miRNAs and eventually influencing the occurrence, proliferation, and metastasis of cancer cells.[105] The ceRNA networks mainly comprise lncRNA-miRNA-mRNA networks and circRNA-miRNA-mRNA networks. Functionally, overexpression of oncogenic lncRNAs/circRNAs against the expression of tumor-suppressive miRNAs can promote tumorous cell proliferation and metastasis, and overexpression of tumor-suppressive lncRNAs/circRNAs restrains the expression of oncogenic miRNAs and thus inhibits carcinogenesis (Figure 1).[105]

Techniques for Identifying lncRNAs and circRNAs

Previous techniques (eg, RNA microarray and mRNA-Seq) used to identify mRNA relied on poly(A) tails and tended to favor the detection of overexpressed transcripts; as such, they have challenges in identifying and analyzing lncRNAs due to their low expression level and poly(A) tail-free properties. In this context, many innovative techniques have been developed for this purpose (Table 3).[106-114] Among them, RNA sequencing, especially transcriptome-wide RNA sequencing, is one of the most commonly used methods.

The special circular structure of circRNAs gives rise to significantly fewer enriched binding sites of RNA binding proteins than the corresponding linear mRNAs.[119] Therefore, historical nucleotide sequence-based approaches may not be suitable for identifying circRNAs and analyzing circRNA-protein interactions. RNA pull-down assays and RNA immunoprecipitation are the mainstays in detecting circRNAs and circRNA-protein interactions (Table 3).[115,116] RNase protection assays,[117] fluorescence in situ hybridization techniques,[115] and electrophoretic mobility shift assays[118] are also used to determine, profile, and understand the biogenesis and functions of circRNAs.

Dysregulated lncRNA-Induced nCRT Resistance/Sensitivity by Sponging Downstream miRNAs

RNA microarray analysis has revealed the close correlation between some lncRNA-miRNA-mRNA networks and nCRT resistance in LARC patients.[72] The expression levels of 3 lncRNAs (ie, lncRNA-KLF7-1, lncRNA-MAB21L2-1, and LINC00324) in the nCRT-sensitive cohort of LARC patients outperformed those in the nCRT-resistant cohort of LARC patients; applying them as the variable subset to predict nCRT sensitivity showed excellent prognostic performance (accuracy = 90%; Table 1).[31] In rectal cancer tissues and cells, upregulating lncRNA-ROR can increase neoplastic CRT resistance by negatively regulating the activity of the p53/miR-145 pathway; conversely, gene knockdown of lncRNA-ROR decreases cell vitality and promotes apoptosis, creating a high CRT sensitivity within tumors.[120] Radiotherapy-activated lncRNA-OIP5-AS1 upregulates *DYRK1A* mRNA by suppressing miR-369-3p, where the remission of cell viability and the promotion of apoptosis also occur, and finally improves the CRT sensitivity of rectal cancer cells.[121] lncRNA-p21 is underexpressed in rectal cancer tissues and cells but can be overexpressed by radiotherapy.[122] Upregulated lncRNA-p21 leads to increased CRT sensitivity in colorectal cancers by blocking the Wnt/β-catenin signaling pathway and inducing the overexpression of the apoptotic gene *Noxa*.[122] The overexpression of lncRNA-EGOT in rectal cancers induces *ErbB4* mRNA expression by targeting miR-211-5p, therefore hampering apoptosis and facilitating cell proliferation, which are both critical to increased CRT resistance.[123] Unfortunately, there are few studies investigating the correlation between circRNA-miRNA-mRNA networks and nCRT resistance in LARC patients, which is a breakthrough worth anticipating.

Table 4. A 4 × 4 Contingency Table With Affiliated Formulas.

Biomarker	Sensitive cases	Resistant cases	Total
Overexpression	A	B	A + B
Underexpression	C	D	C + D
Total	A + C	B + D	A + B + C + D

Sensitivity = A/(A + C); Specificity = D/(B + D); Positive predictive value = A/(A + B); Negative predictive value = D/(C + D); Accuracy = (A + D)/(A + B + C + D).

Conclusions

To date, studies investigating the involvement of transcriptome RNAs (ie, mRNAs and ncRNAs) in variation-related molecular mechanisms for nCRT resistance/sensitivity in LARC patients are still limited. Specifically, dysregulation inherent in rectal cancer or CRT-induced mRNA dysregulation both influence multiple DDR pathways, thus either driving or curbing the DDR process, which plays a central role in changing nCRT resistance/sensitivity in LARC patients. The expression level of these mRNAs is regulated by their upstream targeted ncRNAs; as such, the ceRNA networks constituted by mRNAs plus ncRNAs appear to have a backbone role in affecting the DDR process and the resultant alteration of nCRT resistance/sensitivity. The presently acknowledged lncRNA-miRNA-mRNA networks were identified by RNA microarray analysis on known RNA sequences, so they are confined and imperfect. Additionally, circRNA-miRNA-mRNA networks warrant further investigation since it is still unclear how they impact nCRT resistance/sensitivity in LARC patients. In order to shed more light on the correlation between ceRNA networks and nCRT resistance in LARC patients, it may be worth applying a myriad of novel biological and experimental technologies.

Search Strategy and Inclusion Criteria

Articles published in English were searched in the PubMed database using the search terms (radiotherapy) OR (radiation

therapy) OR (radiosensitive) OR (radiosensitivity) OR (radioresistant) OR (radioresistance) AND (rectal OR rectum) AND (cancer OR tumor OR tumor OR carcinoma OR neoplasm) AND (RNA OR gene). The publications were retrieved on May 28, 2021. Clinical studies that assessed the dysregulation of mRNAs/ncRNAs affecting nCRT resistance/sensitivity in local advanced rectal cancer patients or experimental articles that investigated the association between dysregulated mRNAs/ncRNAs and CRT resistance/sensitivity in rectal cancer cells met the inclusion criteria.

Statistical Methods

All articles present sensitivity, specificity, and the number of sensitive cases and resistant cases for calculating the prognostic performance of biomarkers (ie, overexpressed ceRNAs, miRNAs, and mRNAs). A 4×4 contingency table with affiliated formulas (shown in Table 4) was constructed to compute the accuracy of biomarkers in the prognosis of neoadjuvant chemoradiotherapy resistance in locally advanced rectal cancer.

Authors' Contributions

LH: writing manuscript, table and figure drawing; HC: writing manuscript; YQ: writing manuscript, supervision; BZ: writing manuscript, data collection; QS: conception/design; final approval of manuscript. All authors reviewed and approved the manuscript prior to submission.

Declaration of Conflicting Interests

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ORCID iD

Lin He https://orcid.org/0000-0002-7390-1702

References

1. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut*. 2017;66(4):683-691.
2. Siegel RL, Miller KD. Cancer statistics, 2020. *CA Cancer J Clin*. 2020;70(1):145-164.
3. Siegel R, Desantis C, Jemal A. Colorectal cancer statistics, 2014. *CA Cancer J Clin*. 2014;64(2):104-117.
4. Li M, Gu J. Changing patterns of colorectal cancer in China over a period of 20 years. *World J Gastroenterol*. 2005;11(30):4685-4688.
5. Xu AG, Yu ZJ, Jiang B, et al. Colorectal cancer in Guangdong Province of China: a demographic and anatomic survey. *World J Gastroenterol*. 2010;16(8):960-965.
6. De Caluwé L, Van Nieuwenhove Y, Ceelen WP. Preoperative chemoradiation versus radiation alone for stage II and III resectable rectal cancer. *Cochrane Database Syst Rev*. 2013:Feb 28;(2):CD006041. doi:10.1002/14651858.CD006041.pub3.
7. Rödel C, Liersch T, Becker H, et al. Preoperative chemoradiotherapy and postoperative chemotherapy with fluorouracil and oxaliplatin versus fluorouracil alone in locally advanced rectal cancer: initial results of the German CAO/ARO/AIO-04 randomised phase 3 trial. *Lancet Oncol*. 2012;13(7):679-687.
8. Yan X, Chen J, Meng Y, He C. RAD18 May function as a predictor of response to preoperative concurrent chemoradiotherapy in patients with locally advanced rectal cancer through caspase-9-caspase-3-dependent apoptotic pathway. *Cancer Med*. 2019;8(6):3094-3104.
9. Fokas E, Liersch T, Fietkau R, et al. Tumor regression grading after preoperative chemoradiotherapy for locally advanced rectal carcinoma revisited: updated results of the CAO/ARO/AIO-94 trial. *J Clin Oncol*. 2014;32(15):1554-1562.
10. Mandard AM, Dalibard F, Mandard JC, et al. Pathologic assessment of tumor regression after preoperative chemoradiotherapy of esophageal carcinoma. Clinicopathologic correlations. *Cancer*. 1994;73(11):2680-2686.
11. Svoboda M, Sana J, Fabian P, et al. MicroRNA expression profile associated with response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer patients. *Radiat Oncol*. 2012;7:195.
12. Morgan MA, Lawrence TS. Molecular pathways: overcoming radiation resistance by targeting DNA damage response pathways. *Clin Cancer Res*. 2015;21(13):2898-2904.
13. Smeenk G, Wiegant WW, Vrolijk H, Solari AP, Pastink A, van Attikum H. The NuRD chromatin-remodeling complex regulates signaling and repair of DNA damage. *J Cell Biol*. 2010;190(5):741-749.
14. Cai Y, Geutjes EJ, de Lint K, et al. The NuRD complex cooperates with DNMTs to maintain silencing of key colorectal tumor suppressor genes. *Oncogene*. 2014;33(17):2157-2168.
15. Wang L, Zhang XM, Li Z, et al. Overexpression of nuclear β-catenin in rectal adenocarcinoma is associated with radioresistance. *World J Gastroenterol*. 2013;19(40):6876-6882.
16. Chang H, Wei JW, Tao YL, et al. CCR6 Is a predicting biomarker of radiosensitivity and potential target of radiosensitization in rectal cancer. *Cancer Res Treat*. 2018;50(4):1203-1213.
17. Ferrandon S, DeVecchio J, Duraes L. CoA synthase (COASY) mediates radiation resistance via PI3 K signaling in rectal cancer. *Cancer Res*. 2020;80(2):334-346.
18. Hongo K, Kazama S, Sunami E, et al. Immunohistochemical detection of CD133 is associated with tumor regression grade after chemoradiotherapy in rectal cancer. *Med Oncol*. 2012;29(4):2849-2857.
19. Wang HC, Chou CL, Yang CC. Over-expression of CHD4 is an independent biomarker of poor prognosis in patients with rectal cancers receiving concurrent chemoradiotherapy. *Int J Mol Sci*. 2019;20(17):4087.
20. Zhang RX, Zhou ZG, Lu SX, et al. Pim-3 as a potential predictor of chemoradiotherapy resistance in locally advanced rectal cancer patients. *Sci Rep*. 2017;7(1):16043.
21. Troncarelli Flores BC, Souza ESV, Ali Abdallah E, Mello CAL, Gobo Silva ML. Molecular and kinetic analyses of circulating tumor cells as predictive markers of treatment response in locally advanced rectal cancer patients. *Cells*. 2019;8(7):641.

22. Huang MY, Huang JJ, Huang CM, et al. Relationship between expression of proteins ERCC1, ERCC2, and XRCC1 and clinical outcomes in patients with rectal cancer treated with FOLFOX-based preoperative chemoradiotherapy. *World J Surg*. 2017;41(11):2884-2897.
23. Zlobec I, Vuong T, Compton CC, et al. Combined analysis of VEGF and EGFR predicts complete tumour response in rectal cancer treated with preoperative radiotherapy. *Br J Cancer*. 2008;98(2):450-456.
24. Caramés C, Cristóbal I, Moreno V, et al. MicroRNA-21 predicts response to preoperative chemoradiotherapy in locally advanced rectal cancer. *Int J Colorectal Dis*. 2015;30(7):899-906.
25. Caramés C, Cristobal I, Moreno V, et al. MicroRNA-31 emerges as a predictive biomarker of pathological response and outcome in locally advanced rectal cancer. *Int J Mol Sci*. 2016;17(6):878.
26. Machackova T, Trachtova K, Prochazka V, et al. Tumor microRNAs identified by small RNA sequencing as potential response predictors in locally advanced rectal cancer patients treated with neoadjuvant chemoradiotherapy. *Cancer Genomics Proteomics*. 2020;17(3):249-257.
27. Lopes-Ramos CM, Habr-Gama A, Quevedo Bde S, et al. Overexpression of miR-21-5p as a predictive marker for complete tumor regression to neoadjuvant chemoradiotherapy in rectal cancer patients. *BMC Med Genomics*. 2014;7:68.
28. Luo J, Liu L, Zhou N, et al. miR-519b-3p promotes responsiveness to preoperative chemoradiotherapy in rectal cancer patients by targeting ARID4B. *Gene*. 2018;655:84-90.
29. Bhangu A, Wood G, Brown G, Darzi A, Tekkis P, Goldin R. The role of epithelial mesenchymal transition and resistance to neoadjuvant therapy in locally advanced rectal cancer. *Colorectal Dis*. 2014;16(4):O133-O143.
30. Hotchi M, Shimada M, Kurita N, et al. microRNA expression is able to predict response to chemoradiotherapy in rectal cancer. *Mol Clin Oncol*. 2013;1(1):137-142.
31. Ferrando L, Cirmena G, Garuti A, et al. Development of a long non-coding RNA signature for prediction of response to neoadjuvant chemoradiotherapy in locally advanced rectal adenocarcinoma. *PLoS One*. 2020;15(2):e0226595.
32. Reed E. Platinum-DNA adduct, nucleotide excision repair and platinum based anti-cancer chemotherapy. *Cancer Treat Rev*. 1998;24(5):331-344.
33. Altaha R, Liang X, Yu JJ, Reed E. Excision repair cross complementing-group 1: gene expression and platinum resistance. *Int J Mol Med*. 2004;14(6):959-970.
34. Weaver DA, Crawford EL, Warner KA, Elkhairi F, Khuder SA, Willey JC. ABCC5, ERCC2, XPA and XRCC1 transcript abundance levels correlate with cisplatin chemoresistance in non-small cell lung cancer cell lines. *Mol Cancer*. 2005;4(1):18.
35. Negrei C, Hudita A, Ginghina O, et al. Colon cancer cells gene expression signature as response to 5-fluorouracil, oxaliplatin, and folinic acid treatment. *Front Pharmacol*. 2016;7:172.
36. Tang M, Lu X, Zhang C, et al. Downregulation of SIRT7 by 5-fluorouracil induces radiosensitivity in human colorectal cancer. *Theranostics*. 2017;7(5):1346-1359.
37. Rimkus C, Friederichs J, Boulesteix AL, et al. Microarray-based prediction of tumor response to neoadjuvant radiochemotherapy of patients with locally advanced rectal cancer. *Clin Gastroenterol Hepatol*. 2008;6(1):53-61.
38. Schmidt-Ullrich RK, Mikkelsen RB, Dent P, et al. Radiation-induced proliferation of the human A431 squamous carcinoma cells is dependent on EGFR tyrosine phosphorylation. *Oncogene*. 1997;15(10):1191-1197.
39. Dittmann K, Mayer C, Kehlbach R, Rodemann HP. Radiation-induced caveolin-1 associated EGFR internalization is linked with nuclear EGFR transport and activation of DNA-PK. *Mol Cancer*. 2008;7:69.
40. Baptistella AR, Landemberger MC, Dias MVS, et al. Rab5C enhances resistance to ionizing radiation in rectal cancer. *J Mol Med (Berl)*. 2019;97(6):855-869.
41. Srinivasan B, Sibon OC. Coenzyme A, more than "just" a metabolic cofactor. *Biochem Soc Trans*. 2014;42(4):1075-1079.
42. Lee SG, Su ZZ, Emdad L, Sarkar D, Fisher PB. Astrocyte elevated gene-1 (AEG-1) is a target gene of oncogenic Ha-ras requiring phosphatidylinositol 3-kinase and c-Myc. *Proc Natl Acad Sci U S A*. 2006;103(46):17390-17395.
43. Lee SG, Su ZZ, Emdad L, Sarkar D, Franke TF, Fisher PB. Astrocyte elevated gene-1 activates cell survival pathways through PI3K-Akt signaling. *Oncogene*. 2008;27(8):1114-1121.
44. Sarkar D, Park ES, Emdad L, Lee SG, Su ZZ, Fisher PB. Molecular basis of nuclear factor-kappaB activation by astrocyte elevated gene-1. *Cancer Res*. 2008;68(5):1478-1484.
45. Yoo BK, Emdad L, Lee SG, et al. Astrocyte elevated gene-1 (AEG-1): a multifunctional regulator of normal and abnormal physiology. *Pharmacol Ther*. 2011;130(1):1-8.
46. Emdad L, Lee SG, Su ZZ, et al. Astrocyte elevated gene-1 (AEG-1) functions as an oncogene and regulates angiogenesis. *Proc Natl Acad Sci U S A*. 2009;106(50):21300-21305.
47. Gnosa S, Zhang H, Brodin VP, Carstensen J, Adell G, Sun XF. AEG-1 expression is an independent prognostic factor in rectal cancer patients with preoperative radiotherapy: a study in a Swedish clinical trial. *Br J Cancer*. 2014;111(1):166-173.
48. Kapur N, Mir H, Clark Iii CE, et al. CCR6 Expression in colon cancer is associated with advanced disease and supports epithelial-to-mesenchymal transition. *Br J Cancer*. 2016;114(12):1343-1351.
49. Nandi B, Pai C, Huang Q, Prabhala RH, Munshi NC, Gold JS. CCR6, The sole receptor for the chemokine CCL20, promotes spontaneous intestinal tumorigenesis. *PLoS One*. 2014;9(5):e97566.
50. Stolina M, Sharma S, Lin Y, et al. Specific inhibition of cyclooxygenase 2 restores antitumor reactivity by altering the balance of IL-10 and IL-12 synthesis. *J Immunol*. 2000;164(1):361-370.
51. Tsujii M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc Natl Acad Sci U S A*. 1997;94(7):3336-3340.
52. Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. *Cell*. 1998;93(5):705-716.
53. Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell*. 1995;83(3):493-501.
54. DuBois RN, Shao J, Tsujii M, Sheng H, Beauchamp RD. G1 delay in cells overexpressing prostaglandin endoperoxide synthase-2. *Cancer Res*. 1996;56(4):733-737.

55. Sheng H, Shao J, Morrow JD, Beauchamp RD, DuBois RN. Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. *Cancer Res*. 1998;58(2):362-366.
56. de Heer P, Gosens MJ, de Bruin EC, et al. Cyclooxygenase 2 expression in rectal cancer is of prognostic significance in patients receiving preoperative radiotherapy. *Clin Cancer Res*. 2007;13(10):2955-2960.
57. Jin R, Liu W, Menezes S, et al. The metastasis suppressor NDRG1 modulates the phosphorylation and nuclear translocation of β-catenin through mechanisms involving FRAT1 and PAK4. *J Cell Sci*. 2014;127(Pt 14):3116-3130.
58. Zhang D, Jia J, Zhao G, Yue M, Yang H, Wang J. NDRG1 Promotes the multidrug resistance of neuroblastoma cells with upregulated expression of drug resistant proteins. *Biomed Pharmacother*. 2015;76:46-51.
59. Kim SC, Shin YK, Kim YA, Jang SG, Ku JL. Identification of genes inducing resistance to ionizing radiation in human rectal cancer cell lines: re-sensitization of radio-resistant rectal cancer cells through down regulating NDRG1. *BMC Cancer*. 2018;18(1):594.
60. Cao H, Xu E, Liu H, Wan L, Lai M. Epithelial-mesenchymal transition in colorectal cancer metastasis: a system review. *Pathol Res Pract*. 2015;211(8):557-569.
61. Shao M, Bi T, Ding W, et al. OCT4 Potentiates radio-resistance and migration activity of rectal cancer cells by improving epithelial-mesenchymal transition in a ZEB1 dependent manner. *Biomed Res Int*. 2018;2018:3424956.
62. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. *Nat Rev Cancer*. 2005;5(4):275-284.
63. Miotto B, Chibi M, Xie P, et al. The RBBP6/ZBTB38/MCM10 axis regulates DNA replication and common fragile site stability. *Cell Rep*. 2014;7(2):575-587.
64. Huang P, Ma X, Zhao Y, Miao L. The C. elegans Homolog of RBBP6 (RBPL-1) regulates fertility through controlling cell proliferation in the germline and nutrient synthesis in the intestine. *PLoS One*. 2013;8(3):e58736.
65. Dlamini Z, Rupnarain C, Naicker S, Hull R, Mbita Z. Expression analysis and association of RBBP6 with apoptosis in colon cancers. *J Mol Histol*. 2016;47(2):169-182.
66. Xiao C, Wang Y, Zheng M, et al. RBBP6 Increases radioresistance and serves as a therapeutic target for preoperative radiotherapy in colorectal cancer. *Cancer Sci*. 2018;109(4):1075-1087.
67. Zhou Z, Zhang R, Wang R, et al. Expression of Pim-3 in colorectal cancer and its relationship with prognosis. *Tumour Biol*. 2016;37(7):9151-9156.
68. Yao YF, Du CZ, Chen N, Chen P, Gu J. Expression of HER-2 in rectal cancers treated with preoperative radiotherapy: a potential biomarker predictive of metastasis. *Dis Colon Rectum*. 2014;57(5):602-607.
69. Harpain F, Ahmed MA, Hudec X, et al. FGF8 Induces therapy resistance in neoadjuvantly radiated rectal cancer. *J Cancer Res Clin Oncol*. 2019;145(1):77-86.
70. Chen TJ, He HL, Shiue YL, et al. High chloride channel accessory 1 expression predicts poor prognoses in patients with rectal cancer receiving chemoradiotherapy. *Int J Med Sci*. 2018;15(11):1171-1178.
71. Wu XY, Wu ZF, Cao QH, et al. Insulin-like growth factor receptor-1 overexpression is associated with poor response of rectal cancers to radiotherapy. *World J Gastroenterol*. 2014;20(43):16268-16274.
72. Li N, Yu J, Luo A, et al. LncRNA and mRNA signatures associated with neoadjuvant chemoradiotherapy downstaging effects in rectal cancer. *J Cell Biochem*. 2019;120(4):5207-5217.
73. Jiang D, Wang X, Wang Y, et al. Mutation in BRAF and SMAD4 associated with resistance to neoadjuvant chemoradiation therapy in locally advanced rectal cancer. *Virchows Arch*. 2019;475(1):39-47.
74. Tan Y, Shao R, Li J, et al. PITPNC1 Fuels radioresistance of rectal cancer by inhibiting reactive oxygen species production. *Ann Transl Med*. 2020;8(4):126.
75. Birkman EM, Elzagheid A, Jokilehto T, et al. Protein phosphatase 2A (PP2A) inhibitor CIP2A indicates resistance to radiotherapy in rectal cancer. *Cancer Med*. 2018;7(3):698-706.
76. Meng WJ, Pathak S, Ding ZY, et al. Special AT-rich sequence binding protein 1 expression correlates with response to preoperative radiotherapy and clinical outcome in rectal cancer. *Cancer Biol Ther*. 2015;16(12):1738-1745.
77. Clifford RE, Govindarajah N, Bowden D, Sutton P, Glenn M, Darvish-Damavandi M. Targeting acid ceramidase to improve the radiosensitivity of rectal cancer. *Cells*. 2020;9(12):2693.
78. Senetta R, Duregon E, Sonetto C, et al. YKL-40/c-Met expression in rectal cancer biopsies predicts tumor regression following neoadjuvant chemoradiotherapy: a multi-institutional study. *PLoS One*. 2015;10(4):e0123759.
79. Lin CY, Tian YF, Wu LC, et al. Rsf-1 expression in rectal cancer: with special emphasis on the independent prognostic value after neoadjuvant chemoradiation. *J Clin Pathol*. 2012;65(8):687-692.
80. Saigusa S, Inoue Y, Tanaka K, et al. Significant correlation between LKB1 and LGR5 gene expression and the association with poor recurrence-free survival in rectal cancer after preoperative chemoradiotherapy. *J Cancer Res Clin Oncol*. 2013;139(1):131-138.
81. Saigusa S, Toiyama Y, Tanaka K, et al. Prognostic significance of glucose transporter-1 (GLUT1) gene expression in rectal cancer after preoperative chemoradiotherapy. *Surg Today*. 2012;42(5):460-469.
82. Sheu MJ, Li CF, Lin CY, et al. Overexpression of ANXA1 confers independent negative prognostic impact in rectal cancers receiving concurrent chemoradiotherapy. *Tumour Biol*. 2014;35(8):7755-7763.
83. Ha YJ, Tak KH, Kim CW, et al. PSMB8 As a candidate marker of responsiveness to preoperative radiation therapy in rectal cancer patients. *Int J Radiat Oncol Biol Phys*. 2017;98(5):1164-1173.
84. Huang A, Xiao Y, Peng C, et al. 53BP1 Expression and immunoscore are associated with the efficacy of neoadjuvant chemoradiotherapy for rectal cancer. *Strahlenther Onkol.* 2020;196(5):465-473.
85. Svoboda M, Izakovicova Holla L, Sefr R, et al. Micro-RNAs miR125b and miR137 are frequently upregulated in response to capecitabine chemoradiotherapy of rectal cancer. *Int J Oncol*. 2008;33(3):541-547.
86. Yu J, Li N, Wang X, et al. Circulating serum microRNA-345 correlates with unfavorable pathological response to preoperative

chemoradiotherapy in locally advanced rectal cancer. *Oncotarget*. 2016;7(39):64233-64243.
87. Li AL, Chung TS, Chan YN, et al. microRNA expression pattern as an ancillary prognostic signature for radiotherapy. *J Transl Med*. 2018;16(1):341.
88. Salendo J, Spitzner M, Kramer F, et al. Identification of a microRNA expression signature for chemoradiosensitivity of colorectal cancer cells, involving miRNAs-320a, −224, −132 and let7 g. *Radiother Oncol*. 2013;108(3):451-457.
89. Agostini M, Zangrando A, Pastrello C, et al. A functional biological network centered on XRCC3: a new possible marker of chemoradiotherapy resistance in rectal cancer patients. *Cancer Biol Ther*. 2015;16(8):1160-1171.
90. Huang X, Taeb S, Jahangiri S, et al. miRNA-95 mediates radioresistance in tumors by targeting the sphingolipid phosphatase SGPP1. *Cancer Res*. 2013;73(23):6972-6986.
91. Zhang P, Wang L, Rodriguez-Aguayo C, et al. miR-205 acts as a tumour radiosensitizer by targeting ZEB1 and Ubc13. *Nat Commun*. 2014;5:5671.
92. Thi HT Ha, Kim HY, Kim YM, Hong S. MicroRNA-130a modulates a radiosensitivity of rectal cancer by targeting SOX4. *Neoplasia*. 2019;21(9):882-892.
93. Kelley KA, Ruhl RA, Rana SR, et al. Understanding and resetting radiation sensitivity in rectal cancer. *Ann Surg*. 2017;266(4):610-616.
94. Winter SF, Lukes L, Walker RC, Welch DR, Hunter KW. Allelic variation and differential expression of the mSIN3A histone deacetylase complex gene Arid4b promote mammary tumor growth and metastasis. *PLoS Genet*. 2012;8(5):e1002735.
95. D'Angelo E, Zanon C, Sensi F, et al. miR-194 as predictive biomarker of responsiveness to neoadjuvant chemoradiotherapy in patients with locally advanced rectal adenocarcinoma. *J Clin Pathol*. 2018;71(4):344-350.
96. Huang X, Fejes Tóth K, Aravin AA. piRNA Biogenesis in Drosophila melanogaster. *Trends Genet*. 2017;33(11):882-894.
97. Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol*. 2009;10(2):126-139.
98. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116(2):281-297.
99. Slaby O, Svoboda M, Michalek J, Vyzula R. MicroRNAs in colorectal cancer: translation of molecular biology into clinical application. *Mol Cancer*. 2009;8:102.
100. Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. *Nat Rev Genet*. 2016;17(1):47-62.
101. Yao RW, Wang Y, Chen LL. Cellular functions of long noncoding RNAs. *Nat Cell Biol*. 2019;21(5):542-551.
102. Thi HT Ha, Duong HQ, Hong S. Emerging roles of non-coding RNAs in the response of rectal cancer to radiotherapy (review). *Int J Oncol*. 2021;58(3):344-358.
103. Li X, Yang L, Chen LL. The biogenesis, functions, and challenges of circular RNAs. *Mol Cell*. 2018;71(3):428-442.
104. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta stone of a hidden RNA language? *Cell*. 2011;146(3):353-358.
105. Han TS, Hur K. Epigenetic associations between lncRNA/circRNA and miRNA in hepatocellular carcinoma. *Cancers (Basel)*. 2020;12(9):2622.
106. Zhang H, Zeitz MJ, Wang H, et al. Long noncoding RNA-mediated intrachromosomal interactions promote imprinting at the Kcnq1 locus. *J Cell Biol*. 2014;204(1):61-75.
107. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet*. 2009;10(1):57-63.
108. Clark MB, Mercer TR, Bussotti G, et al. Quantitative gene profiling of long noncoding RNAs with targeted RNA sequencing. *Nat Methods*. 2015;12(4):339-342.
109. Zhao J, Ohsumi TK, Kung JT, et al. Genome-wide identification of polycomb-associated RNAs by RIP-seq. *Mol Cell*. 2010;40(6):939-953.
110. Chu C, Qu K, Zhong FL, Artandi SE, Chang HY. Genomic maps of long noncoding RNA occupancy reveal principles of RNA-chromatin interactions. *Mol Cell*. 2011;44(4):667-678.
111. Dunagin M, Cabili MN, Rinn J, Raj A. Visualization of lncRNA by single-molecule fluorescence in situ hybridization. *Methods Mol Biol*. 2015;1262:3-19.
112. Underwood JG, Uzilov AV, Katzman S, et al. Fragseq: transcriptome-wide RNA structure probing using high-throughput sequencing. *Nat Methods*. 2010;7(12):995-1001.
113. Han J, Zhang J, Chen L, et al. Efficient in vivo deletion of a large imprinted lncRNA by CRISPR/Cas9. *RNA Biol*. 2014;11(7):829-835.
114. Lee RG, Crosby J, Baker BF, Graham MJ, Crooke RM. Antisense technology: an emerging platform for cardiovascular disease therapeutics. *J Cardiovasc Transl Res*. 2013;6(6):969-980.
115. Du WW, Zhang C, Yang W, Yong T, Awan FM, Yang BB. Identifying and characterizing circRNA-protein interaction. *Theranostics*. 2017;7(17):4183-4191.
116. Huang A, Zheng H, Wu Z, Chen M, Huang Y. Circular RNA-protein interactions: functions, mechanisms, and identification. *Theranostics*. 2020;10(8):3503-3517.
117. Zhao H, Zhu M, Limbo O. RNase H eliminates R-loops that disrupt DNA replication but is nonessential for efficient DSB repair. *EMBO Rep*. 2018;19(5):e45335.
118. Hellman LM, Fried MG. Electrophoretic mobility shift assay (EMSA) for detecting protein-nucleic acid interactions. *Nat Protoc*. 2007;2(8):1849-1861.
119. You X, Vlatkovic I, Babic A, et al. Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity. *Nat Neurosci*. 2015;18(4):603-610.
120. Yang P, Yang Y, An W, et al. The long noncoding RNA-ROR promotes the resistance of radiotherapy for human colorectal cancer cells by targeting the p53/miR-145 pathway. *J Gastroenterol Hepatol*. 2017;32(4):837-845.
121. Zou Y, Yao S, Chen X, et al. LncRNA OIP5-AS1 regulates radioresistance by targeting DYRK1A through miR-369-3p in colorectal cancer cells. *Eur J Cell Biol*. 2018;97(5):369-378.
122. Wang G, Li Z, Zhao Q, et al. LincRNA-p21 enhances the sensitivity of radiotherapy for human colorectal cancer by targeting the Wnt/β-catenin signaling pathway. *Oncol Rep*. 2014;31(4): 1839-1845.
123. Li C, Liu H, Wei R, et al. LncRNA EGOT/miR-211-5p affected radiosensitivity of rectal cancer by competitively regulating ErbB4. *Onco Targets Ther*. 2021;14:2867-2878.