

Dear editors,

Thank you very much for your constructive suggestions, which improved our manuscript significantly.

We have made related revisions according to your suggestions and provided a point-by-point response to the comments as follows:

Sincerely,

Xiao Li

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Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Very good)

Conclusion: Minor revision

Specific Comments to Authors:

Title: The molecular mechanism of non-coding RNAs-mediated radiosensitivity regulation in Colorectal Cancer Xiao Li, Xiuxia Hao, Ruiqing Zhu, and Hongwei Zhou. Comments In this review manuscript, the authors address the role of noncoding RNAs in the context of radiotherapy for colorectal cancer. The manuscript initially outlines the molecular mechanisms that influence both the efficacy and resistance of radiotherapy in colorectal cancer. Subsequently, the authors concisely present the potential functions of noncoding RNAs as diagnostic and predictive biomarkers, as well as therapeutic targets, framed within the context of the aforementioned mechanisms. The discussion is structured according to the three principal categories of noncoding RNAs: microRNAs, long noncoding RNAs, and circular RNAs. It should be noted, however, that radiotherapy is not generally regarded as a major therapeutic modality in the management of colorectal cancer. As a result, the clinical relevance of radiotherapy in this specific disease setting remains limited, making it somewhat difficult to envision practical scenarios in which the knowledge summarized here could be directly applied. The following are several concerns that the authors may wish to address. Major concerns: 1. Because the radiosensitivity of adenocarcinoma is relatively low and the intestinal wall is highly vulnerable to irradiation, a therapeutic dose of radiation can cause devastating injury to the intestine. Under these circumstances, the clinical relevance of radiotherapy in this disease setting remains limited, making it difficult to envision practical scenarios in which the knowledge summarized in this manuscript could be directly applied. Since the relationship between noncoding RNAs and radiosensitivity is primarily mediated through DNA damage repair mechanisms, the review would be

more straightforward if it focused on summarizing the role of noncoding RNAs in the pathogenesis of colorectal cancer rather than their role in radiotherapy. Nevertheless, the attempt to consolidate and critically review the current understanding of noncoding RNAs in colorectal cancer is both timely and valuable. The authors should provide clearer articulation of the manuscript's focus and clinical implications. Minor concerns: 1. Please provide appropriate references to support the statement that circular RNAs play dual functional roles, acting either as competitive endogenous RNAs or as scaffolds for RNA-protein interactions. 2. The authors are encouraged to summarize the functions of noncoding RNAs in a table, as this would greatly facilitate the organization and presentation of the current knowledge.

**Answers: Thank you very much for your suggestions on improving the manuscript.**

1. Radiotherapy is among the most important treatments for colorectal cancer in combination with surgery and/or chemotherapy. The indications are as follows:

Preoperative neoadjuvant radiotherapy/synthetic radiochemotherapy is used to increase the resectability rate and preservation rate of the tumor (such as stage I-III rectal cancer). Adjuvant radiotherapy for high-risk factors after surgery (positive surgical margin, lymph node metastasis). Radical radiotherapy for those who are not eligible for surgery or have recurrence after surgery (mainly long-course synthetic radiochemotherapy). Simple radiotherapy for palliative purposes, to relieve pain or bleeding.

Although adjuvant RT is restricted to rectal cancer, its use is expanding. And biomarkers that predict radiation response are clinically urgent.

Furthermore, the future development directions of radiotherapy in the field of colorectal cancer, such as precision treatment, intelligent planning, and combined immunotherapy, are constantly advancing towards greater safety and effectiveness.

Therefore, we have summarized this article, hoping to provide assistance for everyone's understanding of the related fields.

(References: 1. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Rectal Cancer Version 2.2025; 2. CACA guidelines for holistic integrative management of rectal cancer (2024 Edition) [J]. Chin J Colorec Dis (Electronic Edition), 2025, 14(1): 1-19. DOI:10.3877/cma.j.issn.2095-3224.2025.01.001.)

2. We fully understand that "focusing on the pathogenesis of colorectal cancer" can indeed make the review look concise and clear. However, here I would also like to add a little to explain why we still tend to retain the specific scenario of "radiotherapy" rather than completely replacing it with a generalized tumorigenesis mechanism.

Firstly, as you have pointed out, the relationship between non-coding RNA (ncRNA) and DNA damage repair (DDR) is indeed very close, and DDR itself is a core determinant of radiosensitivity. This precisely provides us with an entry point that can be accurately quantified both experimentally and clinically: under radiation conditions, the "dose-time window" of DNA damage is controllable, which enables us to more clearly define which ncRNAs are truly involved in DDR, rather than merely playing the role of oncogenes or tumor suppressors in cell proliferation or apoptosis.

Secondly, we also strongly agree with the significance of the "pathogenesis" perspective. In fact, in the article, we mentioned the fundamental roles of some ncRNAs in the occurrence and development of CRC, such as proliferation and apoptosis, so that readers can quickly establish a comparison between "general functions" and "radiation-specific functions". In other words, we are not ignoring the pathogenesis, but rather hope to further clarify which mechanisms are "reprogrammed" or "limited" under radiation induction conditions, thereby providing a basis for subsequent precise intervention.

We also fully agree that if not handled properly, the term "radiation therapy" might make some readers feel too distant from daily practice. Therefore, in the

revised version, we have made the following changes:

1) Add a transitional paragraph in the **FUNCTIONS AND ROLES OF NCRNAS** section, stating "from the pathogenesis to the radiotherapy mechanism".

2) Additional tables have been added, enabling readers to clearly see the regulatory effect of ncRNAs on radiotherapy for colorectal cancer at a glance.

3. The clinical application of ncRNAs in regarding the radiosensitivity of colorectal cancer is summarized and discussed in the manuscript. However, this part of the research content is relatively scarce. In the future, we will continue to pay attention to the research papers related to the newly published content and conduct timely summaries and organization.

4. We have added appropriate references to support the statement that circRNAs play dual functional roles, acting as competitive endogenous RNAs or scaffolds for RNA–protein interactions.

1) Yang L, Wilusz JE, Chen LL. Biogenesis and Regulatory Roles of Circular RNAs. *Annu Rev Cell Dev Biol.* 2022 Oct 6;38:263-289. doi: 10.1146/annurev-cellbio-120420-125117. Epub 2022 May 24. PMID: 35609906; PMCID: PMC10119891.

“circRNAs can also act as important scaffolds in the nucleus and the mitochondrion. circPOK has been proposed to interact with and activate the ILF2/3 complex in the nucleus, promoting the transcription of ILF2/3-regulated proliferative and pro-angiogenic factors in the context of mesenchymal tumor progression (Guarnerio et al. 2019).”

2) Hong M, Huang X, Zhu H, Ma J, Li F. The role of circular RNA in immune response to tuberculosis and its potential as a biomarker and therapeutic target. *Front Immunol.* 2025 Apr 16;16:1542686. doi: 10.3389/fimmu.2025.1542686. PMID: 40308608; PMCID: PMC12040640.

“circRNA can also interact with RNA-binding proteins (RBPs) to form complexes that regulate the stability, splicing, and translation of mRNA (32, 44). Circular RNA can also serve as a protein scaffold, mediating interactions

between two proteins or mRNA, and then forming a complex to exert its function (45, 46).”

5. We have organized the contents related to ncRNAs and the radiosensitivity of colorectal cancer into three tables: miRNA and its radiosensitivity in colorectal cancer, lncRNA and its radiosensitivity in colorectal cancer, and circRNA and its radio-sensitivity in colorectal cancer.

Title: The molecular mechanism of non-coding RNAs-mediated radiosensitivity regulation in Colorectal Cancer Xiao Li, Xiuxia Hao, Ruiqing Zhu, and Hongwei Zhou. Comments The authors have replied to my rebuttals; however, I still believe that it would be more straightforward—particularly for clinicians—to emphasize the role of noncoding RNAs in colorectal cancer pathogenesis through their involvement in DNA repair, since radiation therapy is generally considered only for rectal cancer, as the authors themselves have cited in the guidelines. Nevertheless, the manuscript presents significant information and has sufficient impact to stimulate further engagement regarding the role of noncoding RNAs within the colorectal cancer research community.

Reply: Thanks for your comments.