Response to review.

Ms. Ref. Number: 64907
Article title: Poly ADP-ribosylation, a promising target for colorectal cancer treatment

First of all, we would like to thank the editor and expert reviewer for the constructive instructions and comments on our manuscript. We prepared a separate reference list to respond to the instructions and comments.

Science editor:

Issues raised: (1) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor; and (2) Please obtain permission for the use of picture(s). If an author of a submission is re-using a figure or figures published elsewhere, or that is copyrighted, the author must provide documentation that the previous publisher or copyright holder has given permission for the figure to be re-published; and correctly indicating the reference source and copyrights.

Authors' response: The figures are our work. The original figures prepared using PowerPoint were submitted.

Reviewer #1:

Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Minor revision
Specific Comments to Authors: In this manuscript, the author summarized the pivotal role of PARP1 and PARylation in CRC therapy. This review demonstrated that PARP1 plays an important role in DNA repair, maintenance of genomic integrity, and regulation of a variety of metabolic and signal transduction processes. It can be the potential therapeutic target in CRC which has valuable clinical prospect. But there are still several shortcomings.

1. The authors suggested that PARP1 plays a role in DNA damage repair, mitochondrial ROS, and transcriptional regulation. But in what process does the PARP1 or PARylation effects more, or in what states does it play a dominant role? In this paper, the functions of PARP-1 are listed, but the internal relations are not deeply explored, which is not logical enough.

Authors' response: Thank you for the comments. Research on PARP-1 and PARylation in CRC is still in its infancy, therefore it is difficult to present all specific mechanisms. Even so, logically convincing mechanisms were provided for each subject.

DNA damage response and defense mechanisms

"PARP-1 functionally interacts with the DNA single-strand break (SSB) repair factor named X-ray repair cross-complementing protein 1 (XRCC1) which plays an important role in the SSB repair signaling pathway, thus facilitating the recruitment and assembly of the SSB repair machinery. Recent studies have shown that PARylation is induced directly on the BRCA1 C-terminal domain of XRCC1 and mediates the early recruitment of XRCC1 targeting DNA lesions."

"However, cancers that arise from BRCA1 germline mutations are deficient in HR DNA repair and are vulnerable to DNA damage. If DNA lesions are detected in BRCA1-mutated cancers, PARP-1 and PARylation may play a pioneering role in constructing a platform for recruiting NHEJ repair factors, such as DNA-dependent protein kinases."

"PARP-1 and PARylation remove the negative aspects of oxidative stress and exert their key roles in areas of positive utilization related to cancer cell growth or oncogene expression [15, 41]. Antioxidant enzymes are dependent on the activation of the transcriptional action of nuclear factor erythroid-related factor 2 (NRF 2), a basic leucine zipper protein, and NRF2 is involved in maintaining intracellular homeostasis in response to physiological changes between intracellular redox actions [42]. The dissociation of NRF2 and Kelch-like ECH-related protein 1 is promoted as the production of intracellular ROS increases to levels that threaten the survival of CRC cells [43]. It can enhance a wide range of downstream cellular defense processes regulated by NRF2, such as glutamate-cysteine ligase and glutathione S-transferase [42]. Recent studies have revealed molecular cooperation between NRF2 and PARP-1 in the transcription of antioxidant genes [41]. Evidence that PARylation is directly involved in this cooperative process is not yet available; however, the relevance of PARylation in the mechanism of action of Sirtuin 6 related to the transcriptional activity of NRF2 is well demonstrated [41, 44]. In particular, PARP-1 can act by directly binding to the antioxidant response element (ARE) or the promoter of a small Maf heterodimer; therefore, PARylation can be anticipated to play a direct or indirect role in NRF2 activity [41]. Furthermore, counteracting mechanism with PARP-1 and PARylation is denoted by its interaction with the protein kinase B (AKT) pathway. Phosphatidylinositol 3 phosphorylates AKT to induce an active form and acts as a redox sensor in cancer cells [7]. Active AKT contributes to hydrogen peroxide accumulation by stimulating oxidative metabolism and inhibition of class O of forkhead box-dependent catalase; however, PARP-1 and PARylation can inhibit the mammalian target of rapamycin complex 1 signaling pathway, thus resulting in downregulation of AKT activity [7, 45]. At this point, it can be emphasized that PARP-1 and PARylation can directly participate in DNA repair and can maintain redox homeostasis to prevent DNA damage by regulating the oxidation state caused by the rapid growth of CRC."

Chromosomal instability

"This is a promising discovery that metastatic CRC is made inherently resistant to anticancer mechanisms by a taxane, and thereafter, various studies have supported that PARP-1 and PARylation play key roles in such resistance. An important implication in recent studies is that the role of PARP-1 and PARylation in chromosomal instability can be emphasized in the chromatic structure change and regulation of epigenetic genes and mitosis."

"Regulation of chromatin structure by PARP-1 may involve direct binding to histones as well as non-histone proteins or chromatin-related proteins or the alteration of nucleosomal structure through PARylation. It has been demonstrated that environmental stimulation for the development of cancer can induce PARP-1- and PARylation-dependent nucleosome loosening, leading to histone removal and opening of chromatin structures. Activation of PARP-1 promotes chromatin decondensation in response to signaling pathways for cancer cell growth and differentiation. Chromatin decondensation could be induced by competitive displacement of histone H1 in the nucleosomes by PARP-1 and ADP-ribosylation on histone H1. The induction of negatively charged PARylation on histone proteins can reportedly lead to repulsion with DNA, thus leading to chromatin decondensation. Then, PARP-1 activity on chromatin can target a wide range of domains, and at the nucleosomal level, it recognizes specific structural features and binds directly to the nucleosomes. The histone cores of the nucleosomes, such as H2A, H2B, H3, and H4, and the linker histone H1 are well-known direct targets of PARP-1, and such a function of action can be considered as a proof to induce localized decondensation of chromatin. Recent studies indicated that PARP-1 binds to mononucleosomes and interacts with trinucleosomes, which is consistent with its role as a chromatin architectural protein. Thereby, the reduction in affinity for surrounding proteins caused by PARP-1 and PARylation may help protect the linker DNA from nuclease digestion; in this context, its role in the facilitation of the reassembly of free histones into nucleosomes may suggest that PARP-1 and PARylation also act as a chaperone for histone protection under chromosomal instability. Studies on CRC have demonstrated a role of PARylation in the regulation of chromatin relaxation by histone proteins H1, H2A, and H2B."

Modulation of tumor suppressor gene and oncogene expression

"since the current understanding of PARP1-induced PARylation can be emphasized owing to its potential involvement in transcriptional regulation by interaction with PARylated proteins, it is necessary to give an eye to the function of PARP-1 and PARylation concerning the gene regulation of APC, p53, and KRAS in CRC."

"PARP-1 interacts with the T-cell factor 4 in CRC to act as a bridge for the complex interaction of T-cell factor 4 with β-catenin. Through this function, PARP-1 increases the transcriptional activation of T-cell factor 4 and lymphoid enhancer factor with β-catenin. mRNA and protein expression level of PARP-1 is reportedly elevated in the clinical biopsy of familial adenomatous polyposis and sporadic CRC, suggesting that they may be a possible cause of PARP-1 regulatory transcriptional activation in CRC [84]. It has also been demonstrated that PARP-

1-mediated transcription up-regulation with T-cell factor 4 and lymphoid enhancer factor may be increased in sporadic CRC compared to normal tissues. A direct correlation of PARylation with T-cell factor 4 or lymphatic system enhancer has not yet been established; however, it is possible to deduce that transcriptional regulation of PARP-1 is carried out in conjunction with PARylation based on the evidence for PAR accumulation in the nucleus of CRC cells. That is, PARP-1 can positively regulate the transcriptional activity of T-cell factor 4 and lymphoid enhancer factor in CRC, and it can be inferred that APC may be more active in CRC when PARP-1 and PARylation are actively involved. PARP-1 also has a unique function that allows direct regulation of sequence-specific transcription factors, and it can form a complex that allows down-regulation of all transcription processes involving p53. The formation of a transcription inhibitory complex is made possible by direct covalent binding of PAR to p53 to induce p53 stabilization. PARylation of p53 first leads to recruitment of histone deacetylases; this transcriptional inhibitory complex can upregulate cancer-related genes and phenotypes by raising the level of expression of hypoxia-inducing factor-1a and vascular endothelial growth factor, which is related to malignant transformation of CRC. It has been suggested that PARP-1 interacts with the G4 motif region of the KRAS promoter under the tumor microenvironment subjected to oxidative stress, such as increased ROS levels. As aforementioned, oxidative stress caused by ROS can play an important role in the regulation of genetic changes and can be considered a common feature in most solid cancers, particularly contributing to the growth, survival, and metastasis of CRC. Under such a condition, it has been proved that PARP-1 is recruited to the KRAS promoter G4 structure after which it undergoes auto-PARylation. The results revealed the mobilization of the transcription factors, heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1), and the MYC-associated zinc finger protein, as well as the formation of a transcription pre-initiation complex. It may be characterized by favoring recruitment to the promoter of cationic transcription factors required for KRAS transcription, such as HNRNPA1 and MYC-associated zinc finger protein, because of the strong anionic properties of PAR."

2. In the "Non-clinical and clinical studies on CRC treatment" part, the detailed description of PARP1 inhibitor for breast/ovarian/pancreatic cancer therapy can be reduced or deleted.

Authors' response: Thank you for the comments. Following the reviewer's instruction, the contents have been modified.

3. In addition to the five PARP1 inhibitors mentioned in the manuscript, several candidates are currently in clinical studies. The authors can update and supplement other clinical trials related to PARP1 inhibitors, including monotherapy and combination therapy.

Authors' response: Thank you for the comments. Following the reviewer's comments, we have found rucaparib clinical case and added them to Table 1 and the related

paragraph. Among the clinical studies with PARP inhibitors, there were cases of talazoparib and veliparib that were not effective on colorectal cancer, and this is presented in the text. Clinical trials targeting colorectal cancer are currently being conducted on olaparib, niraparib, and rucaparib.

4. In the conclusion part, the application prospects of PARP-1 and PARylation in CRC therapy should be strengthed.

Authors' response: Thank you for the comments. The contents of the conclusion have been supplemented.

5.There are some writing errors in context.

Authors' response: Thank you for the comments. We have done an entire review of the manuscript.