The point-by-point responses to the reviewers' comments

Responses to comments from the Referee 2

Brief summary. This article describes the regulation of FEN1 (Flap endonuclease 1) expression under oxidative stress during DNA base excision repair (BER) by a RNA G-quadruplex (rG4) within its mRNA 5' untranslated region (UTR). The important of this RNA secondary structure and stability is highlighted. The authors propose a model of regulation based on the interaction of hnRNPA1 (heterogeneous nuclear ribonucleoportein in RNA binding protein) to FEN1 rG4. They have used a combination of complementary methods, from cellular assays to biochemistry and biophysics measurements to validate their findings.

In the discussion, an explanation of the possible mechanism of hnRNAPA1 – FEN1 interaction under oxidative stress is missing and it would be helpful.

We feel great thanks for your professional review work on our article. As you are concerned, there are several problems that need to be addressed. According to your nice suggestions, we have made corrections to our previous draft, the detailed corrections are listed below.

Specific comments:

In a few figures, the characters are too small to read: Fig. 2B, Fig. 3A, Fig. 4B and 4C

Thank you for your suggestion. We have enlarged the characters in corresponding figures for better reading. (Figure 2B, Figure 3A, Figures 4B, 4C and 4D)

Fig. 1E. Downregulation effects not clear in this western blot. Is it significant?

Yes. In figure 1D, the downregulation of FEN1 was significant and the P value were 0.0048 (NCI-H460), 0.000034 (SW480), 0.003784 (HCT116) and 0.000155 (MCF-7). We have performed several replicate experiments to confirm this result. And there is a quantitative analysis of FEN1 expression in figure 1E, which facilitates a clearer observation of the downregulation of FEN1 under oxidative stress. (Figures 1C and 1D)

Fig. 3G. FEN1 protein changes under different concentration of TMPyP4 not clear in this western blot. Is it significant?

Yes. The downregulation of FEN1 under different concentrations of TMPyP4 treatment was significant and P value were 0.4654 (0-5 μM), 0.0141 (0-10 μM), 0.0028 (0-20 μM), 0.000004 (0-50 μM) and 0.000003 (0-100 μM). Meanwhile, we quantified the expression of FEN1 in Figure 3I. (Figures 3H and 3I)

It would be more comforting to use an orthogonal method to validate the direct interaction of FEN1 rG4 with hnRNPA1 in solution.

Thank you for your good suggestion. Indeed, our conclusion would be further confirmed if the direct interaction of FEN1 rG4 with hnRNPA1 in solution was verified by using an orthogonal approach. In the orthogonal experiments we encountered some difficulties. However, we also used immunofluorescence experiments to further support our conclusion. The specific experimental steps refer to this report (Liu G, Du W, Sang X, et al. RNA G-quadruplex in TMPRSS2 reduces SARS-CoV-2 infection. Nat Commun. 2022;13(1):1444). rG4 labeled with FAM was transfected into the cells. After 24 hours, cells were fixed, permeabilized and incubated with hnRNPA1 antibodies, followed by binding with fluorescent secondary antibodies. The result showed that rG4 and hnRNPA1 co-localized in MCF-7 cells, which also proved the binding of rG4 and hnRNPA1. The IF data is now added in the revised version. (Figure 4H)