Peer Review File

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Reviewer Comments

It is well known that E2F1 is a critical modulator of cellular senescence in human cancers. Moreover, inhibition of cellular senescence may promote drug resistance in tumor cells. Therefore, Gao et al. have taken up very important topic in their work entitled: “E2F1 inhibits cellular senescence and attenuates Oxaliplatin resistance in colorectal cancer”. The aim of their studies was to investigate whether the regulation of cellular senescence by E2F1 may influenced on chemoresistance of human colon cancer cells on Oxaliplatin.

All experiments supported the overall hypothesis, the interpretation of the experimental results does not raise any objections. My recommendation is minor revision.

However, I have following comments:

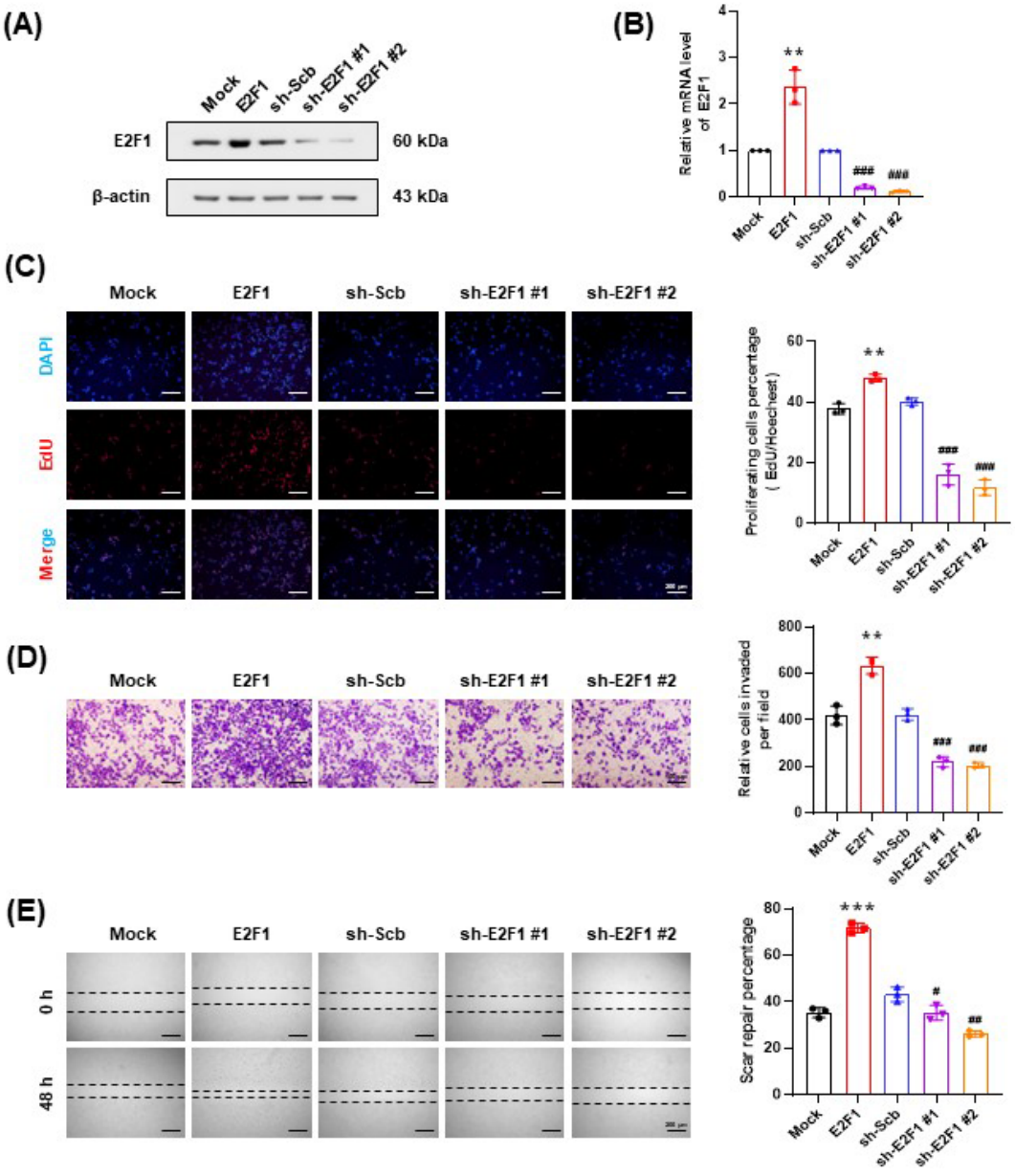
1. Text should be revised for English syntax and grammar. For example: Figure 2C: X-axis: should be percentage.

   Reply 1: Thank you for the suggestions, sorry for the language limitation and grammatical errors. We have carefully edited written English to make the manuscript understandable this time. And we have carefully checked the English labels in the figures to ensure that similar mistakes will not occur again.

   Changes in the text:

   Figure 2C: Y-axis: Changed “perscentage” to “percentage”.

   The tense and grammar errors in the text have been corrected in the document and marked in red font, please check again.



2. Page 2, line 40 – hopefully? – not a scientific term.

Line 55 – "tumor cell were inhibited" – for the general term.

Page 3, line 82 -: can limit unlimited"…?

Reply 2: Thank you for reading carefully. To present our results in a standardized and scientific way, we have re-edited the background section and modified the mistakes you raised in the paper.

Changes in the text:

We have modified our text as advised (see Page 1, lines 27-32; Page 3, lines 46-47; Page 4, lines 72-74).

"**Background:** As a major challenge in the clinical treatment of tumors, tumor drug resistance has always plagued clinicians. Cellular senescence has been reported to have a strong relationship with tumor drug resistance development. The project aims to find novel regulatory factors involved in the aging process of colorectal cancer cells, and explore the effect of cellular senescence on colorectal cancer drug resistance."

"On the other hand, the proliferation, invasion, and migration of tumor cells were inhibited after the suppression of E2F1 expression."

"Under physiological conditions, cellular senescence as an intrinsic cellular response constrains cell proliferation and prevents tumor development by inducing telomere shortening and cell cycle arrest (12,13)."

3. The authors should perform more detailed discussion on the clinical relevance of their findings.

Reply 3: Thanks for your suggestion, it is very useful for us. To clarify the clinical relevance of this research in greater depth, we have added some content to the Discussion section. We have modified our text as advised (see Page 17, lines 365-374).

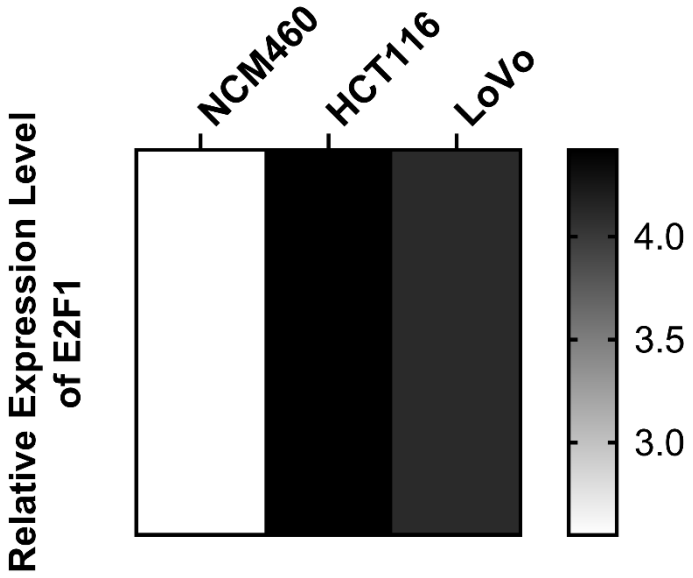
Changes in the text:

"The treatment of tumor patients is difficult, and the emergence of tumor resistance makes this even worse. Combined chemotherapy or the combination of radiotherapy and chemotherapy significantly increases the burden on the patient, and therefore, makes research on tumor drug resistance crucial. Our study demonstrated the significance of E2F1 in the theory of cellular senescence regulating tumor drug resistance, which meant that E2F1 was no longer only a factor regulated by the classical senescence signaling pathway. In contrast, E2F1 will attract more attention as a key target for the aging regulation of drug resistance. The development of targeted drugs for E2F1 will effectively alleviate the treatment dilemma of clinical drug-resistant patients."

4. Too few results on normal NCM460 cell line. What is the level of E2F1 in these cells?

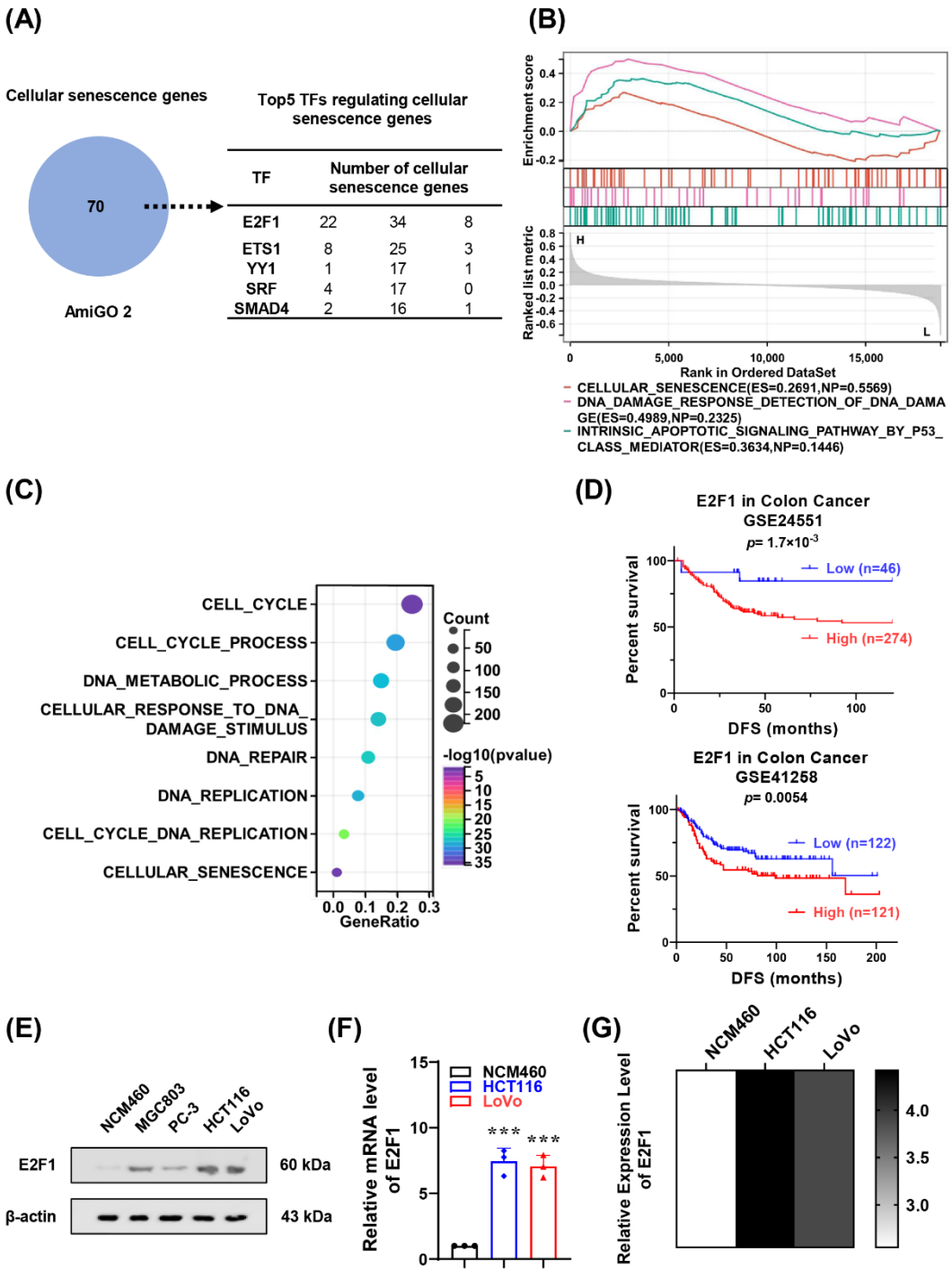
Reply 4: Thank you for your suggestions. In this study, Western blot and qRT-PCR were used to detect the relative expression of E2F1 in normal colon mucosa cell line NCM460, colorectal cancer cell lines HCT116, and LoVo (Figure 1E, F). In addition, according to your comments, we have analyzed the relevant data in the CCLE database and the GEO database (GSM5436026, GSM5436028), and the results showed that the expression level

of E2F1 in the above cell lines was consistent with our experimental results, as shown in the figure below or Figure 1G.



Changes in the text:

We added Figure 1G to Figure 1.



5. I wonder if similar results would have been obtained for irinotecan?

Reply 5: Thanks for this question, it is an interesting proposal, and we can incorporate irinotecan into our future work on related drug-resistance mechanisms. Thank you again for your guidance in this study.