Peer Review File

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Review Comments

Comment 1: I strongly recommend an English review. I have some recommendations that could improve the paper.

Reply 1: We thank Reviewer A for this comment and thank you very much for your careful reading! We have carefully reviewed the English in this manuscript and also asked two native English speakers to help us edit the revision. These recommendations are very helpful and many thanks again.
Changes in the text: We have reviewed carefully in this whole manuscript, and made some modifications. We have kept the track changes in the revised manuscript.

Comment 2: 2. DDR pathways- Although 8 DNA repair pathways are cited in the text, I could not find any explanation or mention for 3 of them (fanconi anemia, check point factors and DNA translesion synthesis) in the text. I suggest that the authors cite only the pathways that they will describe in the manuscript. In addition, direct repair is not among the 8 DNA repair pathways cited, in my opinion it should.

Reply 2: This is an excellent suggestion, and we thank Reviewer A for bringing it up. We agree to cite only the pathways that they will describe in the manuscript since no explanation or mention for fanconi anemia, check point factors and DNA translesion synthesis in the text. So we have modified our text as Reviewer A advised (see Page 6, Lines 96-100). In addition, "direct repair is not among the 8 DNA repair pathways cited, in my opinion it should" is an important point and we thank you for this comment. In response to your comment as well, we have also checked related reports of direct repair and have added this pathway into DDR pathways (see Page 6, Lines 96-100).
Changes in the text: In part 2. DDR Pathways, we have removed the 3 repairs (fanconi anemia, check point factors and DNA translesion synthesis) that were not explained or mentioned in text. We have also included the direct repair. The main components in the DDR system include the direct reversal/repair (DR) pathway, base excision repair (BER) pathway, mismatch repair (MMR) pathway, nucleotide excision repair (NER) pathway, non-homologous end joining (NHEJ) pathway, and homologous recombination repair (HRR) pathway (see Page 6, Lines 96-100).

Comment 3: 2. DDR pathways- There are more MutL homologues. Like MutS, MutL also forms dimers, which are MutLα (MLH1-PMS2), MutLβ (MLH1-MLH3) and MutLγ (MLH1-PMS1). I think that the MutL homologues should be cited in the test.

Reply 3: Thank you for this comment. We have added the MutL homologues mentioned by Reviewer A in our manuscript (see Pages 6-7, Lines 114-116).
Changes in the text: We have added this sentence "There are more MutL homologues. Like MutS, MutL also forms dimers, which are MutLα (MLH1-PMS2), MutLβ (MLH1- PMS1) and MutLγ (MLH1- MLH3)" into the part 2. DDR pathways (see Pages 6-7, Lines 114-116).

Comment 4: 2. DDR pathways- Although HRR is the best one it occurs only during S and G2 phases of the cell cycle, because it depends on the sister chromatid as a template for repair. Thus, NHEJ is the main DSB repair pathway in eukaryotes, even though is an error-prone pathway. It must be clear in the manuscript.

Reply 4: This comment is much appreciated and thank you very much for this helpful suggestion. We have incorporated this suggestion here, agreeing with the comment of Reviewer A (see Page 7, Lines 122-125).
Changes in the text: In part 2. DDR pathways, we have added this sentence: Although HRR is the best one it occurs only during S and G2 phases of the cell cycle, because it depends on the sister chromatid as a template for repair. Thus, NHEJ is the main DSB repair pathway in eukaryotes, even though is an error-prone pathway (see Page 7, Lines 122-125).

Comment 5: 3. Basic Mechanisms of DDR Pathway Deficiency in Cancer- I suggest a review in this phrase: "Isocitrate dehydrogenase-mutant cancers have been showed a DDR deficiency mediated by tricarboxylic acid cycle (TCA) cycle related epigenetic changes (20, 21)." For me it is not clear.

Reply 5: Thank you for the careful review of this sentence. We have checked the original text and amended it (see Page 8, lines 149-154). The isocitrate dehydrogenase (IDH) mutations can induce HRR deficiency, which is mediated by directly inhibiting the α-ketoglutarate (αKG)-dependent dioxygenases, which relates to epigenetic reprogramming in cells. We have modified our text as advised (see Page 8, lines 149-154).
Changes in the text: We have modified the sentence "Isocitrate dehydrogenase-mutant cancers have been showed a DDR deficiency mediated by tricarboxylic acid cycle (TCA) cycle related epigenetic changes (20, 21)" in part 3 to this "Isocitrate dehydrogenase (IDH) mutations have been revealed to induce HRR deficiency mediated by directly inhibiting the α-ketoglutarate (αKG)-dependent dioxygenases (particularly KDM4A and KDM4B), which relates to epigenetic reprogramming in cells" (see Page 8, lines 149-154).

Comment 6: 4. Features of DDR Pathway Alterations in Cancer- Only 4 pathways are detailed in this section. What about the others? If there isn't any protein related to cancer in the other pathways it must be clear.

Reply 6: This is an important point and we thank you for this comment. We are very interested in and fully agree the excellent suggestions of Reviewer A and therefore we conducted a further study about the proteins related to cancer in other pathways. In part 4 "Features of DDR Pathway Alterations in Cancer", we have illustrated some cancer-associated proteins in the direct repair pathway (see Page 9, lines 156-169) and the NHEJ pathway (see Pages 11-12, lines 211-222) respectively.
Changes in the text: According to Reviewer A's comment, we have added the part "4.1 Alterations of the DR pathway: DDR alterations correlate closely with various cancers (Table.1). Epigenetic silencing is the prevalent alteration of the DR pathway……. Thus, these alterations of DR pathway may identify a subset of patients responding to immunotherapies" (see Page 9, lines 156-169) to clarify the alterations of direct repair pathway and cancer. In addition, the details of the NHEJ pathway aberrations and cancer have been added in the part 4.5: "Few defects of the NHEJ pathway have been demonstrated associated with cancers. In the NHEJ pathway, the main proteins mediated DSB repair include XRCC5 (Ku80), XRCC6 (Ku70), XRCC4, DNA Ligase 4 and XLF (NEHJ1) (41) …… The NHEJ polymorphic variants (in particular *LIG4* rs1805388) were reported able to modulate the risk of the radiation pneumonitis in non-small cell lung cancer (NSCLC) patients treated with radiotherapy (41)" (see Pages 11-12, lines 211-222).

Comment 7: 4.1. MMR deficiency- What about the MMR genes related to cancer? I think they must be cited.

Reply 7: Thank you for this suggestion. We have included the MMR genes related to cancer in this part (see Page 10, lines 182-184).
Changes in the text: We have cited this "Germline sequencing of the MMR genes (MLH1, MSH2, MSH6, and PMS2) is commonly performed to detect the MMR deficiencies in clinical practice (30)" in our manuscript (see Page 10, lines 182-184).

Comment 8: 4.2. BER pathway defects- This phrase is not very clear: "BER pathway repairs many types of endogenous DNA damage and one of an important component protein called apurinic/apyrimidinic endonuclease 1 (APE1), is responsible for more than 95% in this process." Although APE1 is a central player in BER, there are many other important players involved in this pathway.

Reply 8: Thank you very much for pointing out this. We have made it clear in our manuscript (see Pages 9-10, lines 171-174).
Changes in the text: We have modified this sentence into "BER pathway repairs multiple types of endogenous DNA damage and many important players are involved in this pathway. One of an important component protein called apurinic/apyrimidinic endonuclease 1 (APE1), a central player in BER, is responsible for more than 95% in this process (27)" (see Pages 9-10, lines 171-174).

Comment 9: 4.3. HRR deficiency- HRR uses the sister chromatid as a template for the repair, for sure it uses a lot of homology. Please review this affirmation: “This DNA repair was aptly named mainly because of lots of homology used as bases reproduction templates.”

Reply 9: We have checked the sentence and HHR, and modified this in our text (see Page 12, lines 224-226), thank you for this comment.
Changes in the text: The sentence has been modified: “This DNA repair is aptly named mainly because it finds a lot of homology, usually using the sister chromatid as a template for reproducing lost or damaged bases” (see Page 12, lines 224-226).

Comment 10: 4.4. NER pathway defects- In NER I think that is important to cite the XP genes that are related to Xeroderma pigmentosum, a genetic syndrome characterized by extreme sun sensitivity, which leads to a higher risk of skin cancer.

Reply 10: We agree this comment and have included the content of XP genes in the part 4.4 “NER Pathway Defects” to elaborate the relationship of XP genes and cancer (see Page 11, lines 205-210).
Changes in the text: As Reviewer A advised, we included the content in our manuscript: “Another important member in the NER pathway is the xeroderma pigmentosum (XP) genes (XP-A to -G and variant) that are associated with XP, a genetic syndrome characterized by extreme sun sensitivity, which leads to a higher risk of skin cancer (37). This syndrome is extremely rare in clinic, however, durable and dramatic responses to ICIs have been observed in the nonmelanomatous and melanomatous XP-related skin cancers (39-40)” (see Page 11, lines 205-210).

Comment 11: Figure 1- Change cell circle for cell cycle.

Reply 11: Thank you for this comment. We have corrected it (Figure.1).
Changes in the text: According to the comment of Reviewer A, we have change cell circle for cell cycle (see Figure.1).

Comment 12: Table 1- Lack of information about what genomic alteration in MMR genes are related to cancer.

Reply 12: Thank you for this suggestion. We have corrected it and included the specific genomic alteration in MMR genes (see Table.1).
Changes in the text: In the column “Genomic Alteration” (see Table.1), the specific alterations of MMR genes have been added.

Comment 13: Table 1- I did not understand what the column size means. A better explanation is necessary.

Reply 13: Thank you for this comment, and we didn't make it clear before. The column size in Table.1 refers to the number of all assessable cases enrolled in each study, including cases with or without the genomic alteration in the DDR pathway. We have added the explanation as the "note" under the table (see Table.1)
Changes in the text: We included the note to further explain this: "The column "Size of assessable cases" refers to the number of all assessable cases enrolled in each study, including cases with or without the genomic alteration in the DDR pathway" (see Table.1).

Comment 14: Table 1- A better explanation is also necessary for the column prevalence. What the percentage means?

Reply 14: The prevalence in Table.1 means the proportion of the cases of the genomic alteration observed in all assessable cases. The explanation has been also noted (see Table.1). We appreciated your careful reading of our manuscript and many thanks to you again.
Changes in the text: The column "Prevalence" refers to the proportion of the cases of the genomic alteration observed in all assessable cases.