Author’s response to reviews

Title: Metastatic colorectal cancer and severe hypocalcemia following irinotecan administration in a patient with X-linked agammaglobulinemia: a case report

Authors:

Mingming Li (limingming@smmu.edu.cn)
Wei Chen (chenwei123@smmu.edu.cn)
Xiaomeng Sun (xiaomengsun315@163.com)
Zhipeng Wang (wangzhipeng@smmu.edu.cn)
Xun Zou (zouxun911@163.com)
Hua Wei (weihua@smmu.edu.cn)
Zhan Wang (profoundamir@smmu.edu.cn)
Wansheng Chen (chenwansheng@smmu.edu.cn)

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Author’s response to reviews:

Dear editor:

Thanks very much for all of the important suggestions and comments given by you and both of the reviewers. We have revised the manuscript accordingly and carefully. Could you please review it again? Following are the point-to-point responses to the comments. Please kindly note that we locate our amendments according to the page number in the manuscript alone instead of the page number in the entire PDF file. The former is 7 pages below of the latter. We clarify here to avoid confusion.

Looking forward to hearing from you.

Best regards,

Chen Wansheng
Response to Editor Comments:

1. Please have the text edited by a professional language editing service or a native English speaking colleague. There are many issues with grammar, wording, spelling, and/or punctuation that need to be addressed.

Abstract section, line 5, page 2: “Bcakground” has been changed to “Background”.

Abstract section, line 10, page 2: “on the X chromosome” has been changed to “on X chromosome”.

Abstract section, line 13, page 2: Removed “could” from “mutations could disturb”.

Abstract section, line 16-23, page 2: As reviewer Amit Rawat suggested, we changed “For XLA patients with CRC, because of the low levels of B lymphocytes and immunoglobulins, they are more prone to exogenous stimuli, which results in severe drug-related toxicity (DRT).” to “For occasional cases of CRC have been reported in XLA patients, low levels of B lymphocytes and immunoglobulins induced by congenital immune disorder make them more susceptible to drug-related toxicities (DRT).”.

Abstract section, line 25, page 2: “any possible measurements to avoid DRT” has been changed to “any possible measurement to predict DRT”

Abstract section, line 37-51, page 2: We made a major revision of this paragraph to rewrite irregular expressions and clarify obscure. The original saying was “Whole exome sequencing and therapeutic drug monitoring did not reveal any positive markers of DRT; therefore, standard first-line chemotherapy was applied. After the fifth treatment cycle, the disease progressed. Therefore, the administration of oxaliplatin was changed to irinotecan, which was followed by severe hypocalcemia. The patient died due to metastatic CRC after the eighth treatment cycle.”. The modified content is “Since the whole exome sequencing and therapeutic drug monitoring did not reveal any predictive markers of DRT, we applied standard first-line chemotherapy to the patient. However, progressive disease occurred after the fifth treatment cycle. Therefore, the administration of oxaliplatin was changed to irinotecan as second-line therapy. After that, the patient firstly suffered from severe hypocalcemia and eventually died due to metastatic CRC after the eighth treatment cycle.”

Abstract section, line 57, page 2: We changed “the first Chinese” to “the first written record of a Chinese”.

Abstract section, line 59, page 2 - line11, page 3: We also made a major revision of this paragraph. The original saying is “Based on whole exome sequencing and bioinformatics analysis, somatic mutations of ABCA6, C6, and PAX3 might have contributed to the onset and metastasis of CRC. A number of germline mutations of genes related to calcium metabolism (including CACNA2, etc.) and the administration of irinotecan were speculated to be the
causes of severe hypocalcemia. To avoid severe DRT, the genetic background and therapeutic drug monitoring should be taken into consideration before determining the course of chemotherapy treatment for XLA patients with CRC."

The revised content is "Whole exome sequencing and bioinformatic analysis indicated the somatic mutations in ABCA6, C6 and PAX3 genes might contribute to the early-onset and metastasis CRC. Besides, a number of germline mutations in genes related to calcium metabolism (CACNA2D4, CD36, etc.) and the administration of irinotecan were speculated to be the causes of severe hypocalcemia. We therefore suggested that in order to avoid severe DRT, clinicians should take genetic background and therapeutic drug monitoring into consideration while planning chemotherapy treatment for XLA patients with CRC.".

Background section, line 8, page 4: “genetic disorders” had been changed to “genetic mutations”.

Background section, line 10, page 4: We changed “The BTK gene” to “The human BTK gene”.

Background section, line 12-18, page 4: ” Most entries in BTKbase have been from unrelated families (84%) and unique molecular events (52%).” had been changed to “BTKbase is an up-to-date database compiling 1796 entities showing 917 unique BTK mutations from 1749 individuals, two thirds of which are from unrelated families, while one third are believed to be sporadic cases.”.

Background section, line 20, page 4: Removed the clause “, and each of them could be inherited in a different family”.

Background section, line 22, page 4: We changed “has not yet been fully estimated in China” to “has not been calculated in China yet”.

Background section, line 31, page 4: “diseases of XLA” has been changed to “complications of XLA”.

Background section, line 34, page 4: “persisting diarrhea” has been changed to “persistent diarrhea”.

Background section, line 36, page 4: “certain types of cancers” has been changed to “certain types of cancer”.

Background section, line 38-64, page 4: We made a major revision in this paragraph according to the suggestion from reviewer Amit Rawat. “According to the National Comprehensive Cancer Network (NCCN) guidelines, first-line chemotherapy includes 5-fluorouracil (5-FU)-based drugs. For this type of drug, the most common drug-related toxicities (DRT) are hand-foot syndrome, leukopenia, neutropenia, thrombocytopenia, diarrhea, nausea, and vomiting. For XLA patients with CRC, because of the low levels of B lymphocytes and immunoglobulins, they are more prone to exogenous stimuli, which results in severe DRT.” has been changed to “According to the National Comprehensive Cancer Network (NCCN) guidelines for colon cancer and rectal
cancer, 5-fluorouracil (5-FU)-based drugs are recommended and commonly used for first-line chemotherapy. But patients receiving 5-FU-based chemotherapy, alone or in a combination regimen, may experience drug-related toxicities (DRT) involving hand-foot syndrome, leukopenia, neutropenia, thrombocytopenia, diarrhea, nausea and vomiting. Severe DRT not only leads to an early termination of chemotherapy but also causes safety issues. With previous evidence of lymphopenia being an independent factor associated with first-line chemotherapy induced hematologic toxicities in CRC patients, we assume that XLA patients, who are characterized by low levels of B lymphocytes and immunoglobulins, are more likely to develop DRTs when they are diagnosed with CRC and receive chemotherapy.”.

Background section, line 1, page 5: “any possible measurements to DRT” has been changed to “any possible measurement to predict DRT”.

Case presentation section, line 16, page 5: “timeline” has been changed to “timeline of hospitalization”.

Case presentation section, line 34, page 5: “liver” has been changed to “hepatic”.

Case presentation section, line 46, page 5: “Whole exon exome sequencing” has been changed to “Whole exome sequencing (WES)”.

Case presentation section, line 51, page 5: “Somatic mutations were not found on” has been changed to “Somatic mutation was absent in”.

Case presentation section, line 53, page 5: “Mutations were not found in genes related to the efficacy (or safety) of” has been changed to “There is also no mutation in genes related to efficacy or safety of”.

Case presentation section, line 1, page 6: “was applied” has been changed to “was started”.

Case presentation section, line 18-20, page 6: Two “levels” have both been changed to “level”.

Case presentation section, line 20, page 6: “suggest that the patient” has been changed to “suggested that the XLA patient”.

Case presentation section, line 28, page 6: “any signs of” has been changed to “any sign of”.

Case presentation section, line 50, page 6: “relived” has been changed to “relieved”.

Case presentation section, line 53, page 6: “Immediately before” has been changed to “Prior to”.

Case presentation section, line 58, page 6: “worsened afterward” has been changed to “deteriorated”.

Discussion and conclusions section, line 8, page 7: “variant” has been changed to “mutation”.
Discussion and conclusions section, line 16, page 7: Remove “s” from “tumor-adjacent tissues”.

Discussion and conclusions section, line 18, page 7: “an early onset of CRC” has been changed to “an early-onset CRC”.

Discussion and conclusions section, line 28, page 7: Added “However, ” at the beginning of the sentence.

Discussion and conclusions section, line 38-40, page 7: “the most possible initiators (ABCA6, C6, and PAX3) for CRC” has been changed to “the most convincing candidate CRC-driver mutations in ABCA6, C6, and PAX3”.

Discussion and conclusions section, line 42, page 7: “codes for” has been changed to “encodes”.

Discussion and conclusions section, line 45, page 7: “innate and adaptive immune response” has been changed to “innate and adaptive immune responses”.

Discussion and conclusions section, line 47-60, page 7: As reviewer Amit Rawat suggested, we detailed elaborated the statement of “The mutation of C6 could initiate CRC development through TGFB1.” with “It has been shown that dextran-sulfate-sodium (DSS) induced colitis was aggravated in C6-deficient mice with a series of enhanced production of pro-inflammatory mediators, including IL-1β, IL-6, CXCL-1, CCL-3, TGF-β1 and IL-17F, compared with wild-type mice. In addition, exogenous C6 could ameliorate DSS-induced colitis in C6-deficient mice. Since both colitis and enhanced inflammatory are risk factors of colorectal cancer, the deficiency of C6 may also participate in CRC development.”.

Discussion and conclusions section, line 4-6, page 8: “expressed in the liver, heart, and brain and contributes to” has been changed to “expressed in the liver, heart and brain, contributing to”.

Discussion and conclusions section, line 16, page 8: “during the last two decades” has been changed to “over the past two decades”.

Discussion and conclusions section, line 18, page 8: Added the conjunction “and” before “XLA” clause.

Discussion and conclusions section, line 21, page 8: “to screen for CRC” has been changed to “for CRC screening”.

Discussion and conclusions section, line 21-23, page 8: “patients who are beyond 20 and 30 years of age” has been changed to “patients over the age of 20 and 30”.

Discussion and conclusions section, line 23, page 8: “Most of the XLA patients with gastric and colorectal cancer” has been changed to “As most XLA patients with gastric cancer or CRC”.

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Discussion and conclusions section, line 23, page 8: “Most of the XLA patients with gastric and colorectal cancer” has been changed to “As most XLA patients with gastric cancer or CRC”.
Discussion and conclusions section, line 28, page 8: “it has been speculated” has been changed to “it is speculated”.

Discussion and conclusions section, line 30, page 8: “mutations of genes” has been changed to “mutations in genes”.

Discussion and conclusions section, line 33, page 8: “analysis with genes” has been changed to “analysis of genes”.

Discussion and conclusions section, line 43–45, page 8: “(or hypocalcemia)” has been changed to “such as hypocalcemia”.

Discussion and conclusions section, line 48, page 8: “could” has been changed to “may”.

Discussion and conclusions section, line 60, page 8: We changed “the first Chinese” to “the first written record of a Chinese”.

Discussion and conclusions section, line 1, page 9: “plan” has been changed to “regimen”.

Discussion and conclusions section, line 3, page 9: “bioinformatics analysis” has been changed to “bioinformatic analysis”.

Discussion and conclusions section, line 6, page 9: “may have contributed” has been changed to “may contribute”.

Discussion and conclusions section, line 11, page 9: “jointly” has been changed to “collectively”.

Figure legends section, line 3–14, page 18: As reviewer 2 suggested, we merged figure 1 and 2 and moved crucial description on patient’s clinical record and disease progression to the figure, leaving only brief introduction of the figure content in figure legends. The rewritten legend for the merged figure is as follows: “Figure 1. The timeline, diagnosis and progression focus. (A) The timeline of hospitalization: This patient was diagnosed with XLA at the age of 4. He suffered upper abdominal pain on Oct 2016, and was diagnosed with advanced colorectal cancer with liver metastasis by liver biopsy (B, magnification, x100) and PET-CT scan (C). Abdominal CT scans on Oct 21th 2016 (D) and Jan 9th 2017 (E) showed that the tumor was stabilized after the first two treatment cycles. However, abdominal CT scans on Mar 24th 2017 (F) and May 22th 2017 (G) showed metastatic tumor progressed. Blood calcium/potassium levels (H) and tumor load (I) were recorded throughout all treatment cycles. The dotted lines indicate the starting time of each chemotherapy treatment cycle. The dark dotted line indicates the sixth treatment cycle when oxaliplatin was replaced by irinotecan.”.

2. Please indicate the role of the funding body in the design of the study and collection, analysis, interpretation of data and in writing the manuscript in the Funding section.
We revised the Funding section in line 41-53, page 11. The renewed content is as follows.

“During the whole course of this study, the study design, data collection, data analysis, data interpretation and manuscript preparation were mainly supported by the National International Scientific and Technological Cooperation Program, China (Grant No. 2015DFA31810). Part of the data analysis, which is the therapeutic drug monitoring was supported by the Clinical Science and Technology Innovation Project, Shanghai, China (Grant No. SHDC12015120).”

3. Please add a section "Additional files" (after the References/Figure legends) where you list the following information for each additional/supplementary file in the file inventory:

- File name (e.g. Additional file 1)
- Title of data
- Description of data

We appended a full list of additional files catalogue at last. Please find the Additional files section at line 1-24, page 19.

Response to Reviewer 1:

GENERAL COMMENTS:

Amit Rawat (Reviewer 1): The authors describe a patient with X-linked agammaglobulinemia who developed colorectal cancer and suffered from hypocalcemia following irinotecan administration. I have the following comments about the manuscript.

ADDITIONAL REQUESTS/SUGGESTIONS:

1. In the abstract section, the authors mention that “These mutations could disturb B-cell development, decrease immunoglobulin levels, increase susceptibility to infections or neoplasms, and increase the risk of developing colorectal cancer (CRC).”

BTK gene mutations impair B cell development with a block of maturation in Pre B cell stage. Please remove could from "could disturb".

An increased risk of developing CRC is not a usual manifestation in XLA although it has been reported in some patients. Please modify to Occasional cases of CRC have been reported in XLA
pts. The readers should NOT get an incorrect message that CRC is a usual manifestation of XLA, which is NOT correct.

Abstract section, line 13, page 2: “These mutations could disturb” has been changed to “These mutations disturb”.

Abstract section, line 16-18, page 2: “For patients with CRC” has been changed to “For those occasional cases of CRC have been reported in XLA patients”.

2. For XLA patients with CRC, because of the low levels of B lymphocytes and immunoglobulins, they are more prone to exogenous stimuli, which results in severe drug-related toxicity (DRT)

There is no evidence to suggest that patients with XLA develop drug-related toxicity as they are exposed to exogenous stimuli. This is again mentioned in the background section. Omit at both places.

Thank you for your valuable advice. This is indeed a sentence worth considering. However, we believe that the improper wording in the original expression causes misunderstanding. What we are saying here is that XLA patients have low levels of B lymphocytes and immunoglobulins. Lymphopenia alone is a predictive marker of chemotherapy drug-induced toxicity (DRT). Therefore, we conclude that XLA patients with cancer facing a higher chance of getting DRT when treated with chemotherapy. Detailed revisions are as follows.

Abstract section, line 16-23, page 2: We changed “For XLA patients with CRC, because of the low levels of B lymphocytes and immunoglobulins, they are more prone to exogenous stimuli, which results in severe drug-related toxicity (DRT).” to “For occasional cases of CRC have been reported in XLA patients, low levels of B lymphocytes and immunoglobulins induced by congenital immune disorder make them more susceptible to drug-related toxicities (DRT).”.

Background section, line 51-64, page 4: We made a major revision in this paragraph according to your suggestion. “For XLA patients with CRC, because of the low levels of B lymphocytes and immunoglobulins, they are more prone to exogenous stimuli, which results in severe DRT.” has been changed to “Severe DRT not only leads to an early termination of chemotherapy but also causes safety issues. With previous evidence of lymphopenia being an independent factor associated with first-line chemotherapy induced hematologic toxicities in CRC patients, we assume that XLA patients, who are characterized by low levels of B lymphocytes and immunoglobulins, are more likely to develop DRTs when they are diagnosed with CRC and receive chemotherapy.”.
3. Whole exome sequencing at line 34 pg 6 has incorrectly been mentioned as "whole exon" sequencing. Please correct.

Case presentation/Diagnostic Focus and Assessment section, line 46, page 5: “Whole exon sequencing” has been changed to “Whole exome sequencing”.

4. The authors have suggested that mutations in the complement 6 gene may predispose to CRC through TGFb1. Please elaborate and clarify. One of the references cited in support of this statement is regarding an increased susceptibility to dextran induced colitis and NOT CRC.

We did cite an article relating C6 gene to dextran induced colitis, and colitis is indeed not really CRC. But our assumption here is that colitis itself being a risk factor of CRC is confirmed to be associated with C6 through sets of genes including TGFb1. We may therefore infer that C6 is also functionally related to CRC. We added a paragraph to clarify the logic chain I described above. Detailed revisions are as follows.

Discussion and conclusions section, line 47-60, page 7: We detailed elaborated the statement of “The mutation of C6 could initiate CRC development through TGFB1.” with “It has been shown that dextran-sulfate-sodium (DSS) induced colitis was aggravated in C6-deficient mice with a series of enhanced production of pro-inflammatory mediators, including IL-1β, IL-6, CXCL-1, CCL-3, TGF-β1 and IL-17F, compared with wild-type mice. In addition, exogenous C6 could ameliorate DSS-induced colitis in C6-deficient mice. Since both colitis and enhanced inflammatory are risk factors of colorectal cancer, the deficiency of C6 may also participate in CRC development.”.

Response to Reviewer 2:

GENERAL COMMENTS:

Reviewer 2 (Reviewer 2): The manuscript is readily improved and reads easier and is more clear in its main focus.

ADDITIONAL REQUESTS/SUGGESTIONS:

1. However, the figures should be improved as well as the figure legends (lot of text is now 'hidden' in the legends. Also, the authors should strongly consider merging figure 1 and 2, so the reader is more visually guided through the patient's timeline and this will also clarify the case report. Nonetheless, figure legends should be much shorter. Point 5 is adequately adapted.

Thank you for your further review of this manuscript. We merged figure 1 and 2 as recommended, and moved crucial description on patient’s clinical record and disease progression
to the figure, leaving only brief introduction of the figure content in figure legends. Detailed revisions are as follows.

Figure legends section, line 3-14, page 18: The rewritten legend for the merged figure is as follows: “Figure 1. The timeline, diagnosis and progression focus. (A) The timeline of hospitalization: This patient was diagnosed with XLA at the age of 4. He suffered upper abdominal pain on Oct 2016, and was diagnosed with advanced colorectal cancer with liver metastasis by liver biopsy (B, magnification, x100) and PET-CT scan (C). Abdominal CT scans on Oct 21th 2016 (D) and Jan 9th 2017 (E) showed that the tumor was stabilized after the first two treatment cycles. However, abdominal CT scans on Mar 24th 2017 (F) and May 22th 2017 (G) showed metastatic tumor progressed. Blood calcium/potassium levels (H) and tumor load (I) were recorded throughout all treatment cycles. The dotted lines indicate the starting time of each chemotherapy treatment cycle. The dark dotted line indicates the sixth treatment cycle when oxaliplatin was replaced by irinotecan.”.