Peer Review File

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Reviewer A

Comment:

This is a very interesting and original paper, the figure 4 and 5 are very explanatory and only minor revisions should be introduced.

The authors should describe the correlation between immunity-microbiome and cancer as described by Li Yunshu et al. in "Colitis-associated carcinogenesis: crosstalk between tumors, immune cells and gut microbiota" and by Di Tucci et al. in "Immune-Onco-Microbiome: A New Revolution for Gynecological Cancers".

Reply:

Thank you for your insightful comments and recommendations. We appreciate your guidance and the valuable references you provided These two articles provide insights into the correlation between the immune-microbiome, particularly the gut microbiome, and cancer. We specifically referenced these articles along with other relevant literature on the topic, and we have reworked the section on "**Keyword Citation Burst**".

Our bibliometric analysis reveals that the gut microbiome is a keyword with high burst strength in the research field of "gynecological cancers and the microbiome". Given that the gut serves as the body's largest immune organ, we explored the potential immune mechanisms of the gut microbiota in the occurrence, development, and treatment of gynecological cancers.

Initially, we summarized the research perspectives regarding the gut microbiome and cancer. Subsequently, we listed results from the literature concerning the immune-inflammatory mechanisms of the gut microbiota in gynecological cancers, focusing primarily on cervical and ovarian cancers. We further explored how the gut microbiota influences the efficacy of cancer chemoradiotherapy. Following this, we discussed the impact of the "estrogen-gut microbiome axis" on the development of endometrial cancer. Finally, we explored the potential mechanisms of female reproductive tract microbiota in immune responses. Discussions on the gut microbiome's association with immunotherapy are covered in the "Gut Microbiome & Immune Checkpoint Inhibitors" section.

We appreciate your recommendation of these pertinent works, as they significantly contributed to the enhancement of the manuscript's content and the overall quality of our research.

Changes in the text: The modifications have been made in the revised manuscript in lines 353-411.

Reviewer B

Comment 1: Throughout this manuscript the author refers to the microbiota as the microbiome. The microbiome is the genome of a specific microbiota. The authors should accurately edit the words “microbiome” vs “microbiota” throughout the manuscript.

Reply 1: Thank you for your valuable feedback. We acknowledge the oversight in our use of terminology, and we apologize for any confusion it may have caused.

We have thoroughly reviewed each sentence where the term "microbiome" was used and replace it with the appropriate term "microbiota" as needed.

Changes in the text: For example:

Line 311-313: Cluster 6 makes an outstanding contribution by developing a non-invasive HPV infection prediction technique by combining urine metabolome data with the cervicovaginal microbiota.

Line 335-336: As a result, the local microenvironment's symbiotic equilibrium is disrupted by the instability of microbiota.

Line 355-356: Emerging evidence suggests that the uterine microbiota may impact endometrial structure and function, thereby inducing inflammation or dysbiosis, potentially contributing to adverse birth outcomes and endometrial diseases.

Comment 2: The authors cite that gastric cancer is similar to almost all cervical cancer in that HPV precedes it. However, there is not a consensus in the field that HPV does precede gastric cancer and is in fact a hotly debated issue. Further clarification would be necessary to avoid a gross misrepresentation of the field. Consider Baj et al 2022 (PMCID: PMC9179480) as a starting reference.

Reply 2: Thank you for your insightful comments. We apologize for conveying inappropriate viewpoint that “HPV precedes gastric cancer”. We intended to convey that both gastric cancer and cervical cancer are associated with specific infectious pathogens, serving as the initiating factors in their carcinogenesis. We have rephrased the above points and made revisions to our manuscript (see Line 64-66).

Changes in the text: Line 64-66: Specific microorganisms, such as Helicobacter pylori in gastric carcinoma and Streptococcus bovis in colorectal carcinoma, have been linked to carcinogenesis. Similarly, almost all cervical cancer patients have human papillomavirus (HPV) infection.

Comment 3: In line 305-306, the author states that the uterine microbiome ultimately leads to poor birth outcome. This is inaccurate and misquotes citation #47. Unless the author makes clear that infection or dysbiosis of the endometrial cavity is what may actually precede poor birth outcome, and not simply the existence of a uterine microbiome (as it is currently written now), then this statement, as is, is woefully inaccurate and reduces the impact of this manuscript.

Reply 3: Thank you for your careful review and comment. We incorrectly cited citation 47, leading to ambiguity in the sentence. We have modified our text as advised (see Line 316-318).

Changes in the text: Line 316-318: Emerging evidence suggests that the uterine microbiota may impact endometrial structure and function, thereby inducing inflammation or dysbiosis, potentially contributing to adverse birth outcomes and endometrial diseases.

Comment 4: In Line 352, the author states that according to citation #24, patients with high diversity (whether beta or alpha diversity should also be specified here) need more intervention during Chemoradiation treatment. This is an over statement of El Alam et.al.'s findings. One may infer or expect that to be true, but as it is written now this is not accurate. It should be clarified what El Alam et al found, and what may be an inference of the authors of this manuscript.

Reply 4: Thank you for your kind reminder. We have re-examined the literature by El Alam et al. and clarified that the baseline diversity changes in the gut microbiota during chemoradiation treatment refer specifically to alpha diversity. Additionally, we have deleted the statement "patients with high diversity need more intervention during Chemoradiation treatment" as it was an overinterpretation.

Changes in the text: Line 381-384: El Alam et al. found sustained declines in gut microbiome richness and diversity during pelvic CRT for 58 women with cervical, vaginal, or vulvar cancer. Though alpha diversity returned to baseline after 12 weeks, the structure and composition remain changed.

Comment 5: In lines 381-382, the author states that there has been convincing evidence that the gut microbiome can enhance the therapeutic activity in malignant tumors by modulating immunity. This is a large statement that requires several citations to provide evidence to this claim.

Reply 5: Thank you for pointing that out. We added more citations to support the statement (see Line 421-422).

Changes in the text: Line 421-422: In certain malignant tumors, the gut microbiota has been reported to boost therapeutic efficacy by inhibiting checkpoint molecules, with particularly notable findings in melanoma (78–80).

78. Routy B, Le Chatelier E, Derosa L, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science 2018;359:91–7.

79. Gopalakrishnan V, Spencer CN, Nezi L, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 2018;359:97-103.

80. Baruch EN, Youngster I, Ben-Betzalel G, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. Science 2021;371:602–9.

Comment 6: Format font/capitalization consistently in all Tables.

Reply 6: Thank you for bringing this to our attention. We have standardized the font, font size, and journal format across in all tables in the revised manuscript.

Changes in the text: Table 1 (see Line 691-693); Table 2 (see Line 695-696).

Comment 7: Please clarify if simply any crosstalk between the gut-vaginal microbiome is the CAUSE of gynecological cancer or whether this crosstalk may be a SOURCE of gynecological cancer (line 63).

Reply 7: Thank you for your meticulous review and valuable feedback. We have corrected the wrong "CAUSE" to "SOURCE" (see Line 72).

Changes in the text: Line 70-72: The gut and vaginal microbiome share over 30% of commensal bacteria (14), and the gut-vaginal microbiome axis suggests possible crosstalk as a source of gynecological cancer.

Comment 8: Clarify which microbiome is being referred to in line 64.

Reply 8: Thank you for the request. We have revised the statement (see Line 73).

Changes in the text: Line 72-74: It can be concluded that the microbiome of the gut and FRT are associated with gynecological cancers, providing new possibilities for using the microbial signatures as preventive and therapeutic agents.

Comment 9: Clarify whether "average publication year" in line 179 refers to the average number of publications per year. Otherwise, the sentence remains unclear and adds no useful information.

Reply 9: We appreciate your advice, and we have removed this concept in the revised manuscript. The introduction of this concept was intended to explain the arrangement of the nodes in Figure 4 from the bottom left to the top right. However, in our analysis, we emphasized the size and color of the nodes more, which represent the citation count of journals.

Additionally, the "average publication year" reflects the average research output time for a journal, calculated by dividing the sum of publication years by the number of articles. However, in practical application, it has limitations. For instance, in our study, the average publication years of highly cited journals were very close, divided into half-year intervals from 2020 to 2022, showing no significant differences. As we believe that removing the relevant sentences about this concept is more appropriate.

Changes in the text: See Line 186.

Comment 10: The sentence from lines 182-183 needs a citation.

Reply 10: Thank you for highlighting that. We added citations to support the sentence in lines 190-191 in the revised manuscript.

Changes in the text: Line 190-191: Besides, three high-IF reviews were published in Seminars in Cancer Biology in 2022 (4,17,18).

4. Wahid M, Dar SA, Jawed A, et al. Microbes in gynecologic cancers: Causes or consequences and therapeutic potential. Semin Cancer Biol 2022;86:1179-1189.

17. Haque S, Raina R, Afroze N, et al. Microbial dysbiosis and epigenetics modulation in cancer development – A chemopreventive approach. Semin Cancer Biol 2022;86:666–81.

18. Kyrgiou M, Moscicki AB. Vaginal microbiome and cervical cancer. Semin Cancer Biol 2022;86:189–98.

Comment 11: The sentence from line 357 to 359 is unclear and adds no information as it is currently written. The authors should reevaluate to see if a word or phrase needs to be added or remove to improve the clarity of the information they are trying to relay.

Reply 11: We have removed the mentioned sentence from the manuscript as your suggestion.

In the 'Keyword Citation Burst' section, we have extensively revised the content, providing two specific examples involving intentional modifications of the gut microbiota to intervene in the efficacy of gynecological cancer treatments. One example highlights the improvement of gastrointestinal toxicity through fecal microbiota transplantation in healthy individuals, while the other demonstrates the reversal of cisplatin resistance and overall survival in ovarian cancer mice treated with feces from the control group, as opposed to antibiotic-treated mice.

Finally, we draw the following conclusion that these results somewhat endorse the potential impact of gut-vaginal microbiome crosstalk on gynecological cancer progression, providing a promising therapeutic target.

Changes in the text: See Line 387-393.

Comment 12: The author should clarify if they are referring to the relative or absolute abundance of Bacteriodales in line 391.

Reply 12: We fully agree with your suggestion and we have clarified that in the revised manuscript (see Line 432).

Changes in the text: Line 431-433: Conversely, the study suggested that PD-1 non-responsive patients showed a higher relative abundance of Bacteroidales, species that implicated in the immunostimulatory effects of cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) blockade.

Comment 13: The authors should provide more detailed labels in their figure and figures legend. For example, figure 4b is a table where two columns list years but the purposes of both columns are not clear.

Reply 13: Thank you for the suggestion. We recognized the need for more detailed labels in our figures and figure legends. In the citation burst detection networks of Figures 6 and 7, we have added legends to the numerical values, such as "Year, Strength, Begin, End, and 2012-2022". The specific meanings have been described in the 'Terms related to Bibliometric Analysis' section (see Line 106-109). Specifically, keywords and references are the research factors (nodes in this network). The "Year" represents the first appearance of a node,

corresponding to the starting point of the dark blue line, while the red line indicates the beginning and end of the burst cycle. The "2012-2022" is the coverage period of this bibliometric research. Burst strength reflects the importance of a node. In Figure 7, we have highlighted the years (2020 and 2021, enclosed in black boxes) when citation bursts occurred based on the references, offering a glimpse into future research frontiers.

Changes in the text: see the Figure Section (see Line 697-719).