TRANSLATIONAL CANCER RESEARCH

Peer Review File

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Review Comments

In the manuscript "Xenograft Models Reveals a Panel of Dysregulated Circulating Metabolites and Potential Therapeutic Targets for Colorectal Cancer", the author combined the metabolomics approach with PDX of colorectal cancer to discover a panel of potential diagnostic circulating metabolites. Dysregulated 26 amino acid metabolism was a significant feature of colorectal cancer, and transporter SLC7A5 and SLC1A5 were potential metabolic therapeutic targets.
The hypothesis and its design sound scientifically and the statistical methods used are generally technically acceptable. The manuscript can be published after the following issues are addressed.

Major:
1. Metabolites listed in Table 1 need to be examined:
1) Including but not limited to #44 (D-Lactic acid) and #49 (D-Proline): L form is endogenous and GC-MS cannot differentiate D and L form.
We ignored D or L form of the metabolites when we re-analyze.
2) Including but not limited to #4, #13, #96, etc.: not endogenous
The metabolites were list in Table2 (see Page20-21) which removed the exogenous.
3) Please use the same sig figs/decimal places for threshold and experimental data.
We changed the experimental data to four decimal places and the same threshold (see Page 6, Line 130, Page 7, Line138, Page 8, Line 180, Page 9, Line 187-188, Page 9, Line 196-197).
2. Some FC values were too close to 1 (e.g. 1.051 or 0.919) to say the metabolite was up/down-regulated. These FC values are ambiguous considering ~20% variation of GC-MS.
Regarding criteria of differentially expressed metabolites, the use of unadjusted p-value of 5% is too liberal and may increase the false positive rate in a formal study. Even in a pilot study, two criteria, unadjusted p-value and fold-change, are typically used.
The authors need to provide a justification to use only unadjusted p-value of 5%. Otherwise, the authors may consider redoing analyses using two criteria, within-metabolite adjusted p-value and fold-change (maybe, 5% and 1.2 fold-change).
We re-analyze the experimental data by two criteria, within-metabolite adjusted p-value 5%, and 1.2 fold-change (see Page 6, Line 130, Page 7, Line138, Page 8, Line 180, Page 9, Line 187-188, Page 9, Line 196-197).

3. The authors may state that this is an early stage development or a pilot study in the main text. As well, it would be also good for readers to state several study limitations, especially, regarding the very liberal unadjusted thresholds for marker discovery, some potential markers with small effect size (FC), and the small sample size. By doing so, it may avoid over-interpreting the results.
We gave a statement in the main text (see Page 12, Line 267-270).

Minor:
1. Page 3, Line 65-67: Please cite the following paper
Yuan, F.; Kim, S.; Yin, X.; Zhang, X.; Kato, I., Integrating two-dimensional liquid and gas chromatography mass spectrometry for untargeted colorectal cancer metabolomics: a proof of principle study. Metabolites 2020, 10 (9), 343.
We cited this paper (see Page 4, Line 76).
2. Page 3, Line 77 and Page 4, Line 81: Please specify "XX hospital".
We specified "Zhejiang Cancer Hospital" (see Page 4, Line 89, Page 5, Line 99-100,103-104, Page 13-14, Line 295-296, Page 14, Line 299).
3. Page 4, Line 103: Should add a space between number and unit. The same for entire manuscript.
We added the space between number and unit for entire manuscript.
4. Page 4, Line 104-107: Please briefly describe GC-MS method, as least including extraction of metabolites, derivatization method, and instrumentation. Since that is one of main techniques you used to generate data.
We describe the GC-MS method (see Page 6, Line 115-117).
5. Page 5, Line 108-109: "(orthogonal) partial least squares-discriminant analysis (O)PLS-DA were performed" should be "(orthogonal) partial least squares-discriminant analysis, (O)PLS-DA, were performed"
We modified the mistake (Page 6, Line 123-124).
6. Page 5, Line 111-117: Is "VIP" an abbreviation of "Variable importance in the projection" or "variable influence on projection"? Is threshold "1" or "1.0"?
The threshold is "1". "VIP" is an abbreviation of "Variable importance in the projection".
7. Page 5, Line 108-117: Please briefly describe identification method, at least including database/library, similarity threshold, and software.
We described the details (see Page 6, Line 120-122).
8. Page 6, Line 152: Fig. 2a is "PCA of the treatment group vs control group", not QC samples. Please use the right figure.
The Fig.2a is "PCA of the treatment group vs control group" (see Page 8, Line 172-173).
9. Page 6-7, Line 157-168: How many differentially expressed metabolites? 130 or 140 or 96?

There are 130 differentially expressed metabolites based on P-value <0.05 and there are 96 (changed from 96 to 67 when screening with adj.p-value <0.05, FC >= 1.2 or FC <= 0.83) differentially expressed metabolites when screening with P-value <0.05, FC >= 1.3 or FC <= 0.77 finally. The heatmap and bubble plots were also used with 96 metabolites (changed to 36).

10. Page 7, Line 190 and Page 9, Line 244: Please adjust line alignment.

We adjusted line alignment (Page 10, Line 215, Page 12, Line 267-270).

11. Page 13, Fig 2a, 2b and 3a: Please explain what did “T” and “C” stand for.

We explained the “T” and “C” in Page 19, Line 425-426, Page 20, Line 434.

12. Page 13, Fig 2b: It seem sample size of “T” in OPLS-DA analysis is 8, not 10. Why?

The sample size of T is 10. There's some overlap in the figure.