Dear editor,

Thank you for giving me the opportunity to submit a revised draft of my manuscript titled '***Advance in metabolism and target therapy in breast cancer stem cells***' to ***World Journal of Stem Cells***. We appreciate the time and effort that you and the reviewers have dedicated to providing your valuable feedback on my manuscript. We are grateful to the reviewers for their insightful comments on my paper. We have been able to incorporate changes to reflect most of the suggestions provided by the reviewers.

Here is a point-by-point response to the reviewers' comments and concerns.

**Step 1: Please select to revise this manuscript or not**

**Authors' Response:** We have selected "yes".

**Step 2: Key points of revising the manuscript**

**(1) Scientific quality:** Please resolve all issues in the manuscript based on the peer review report and make a point-to-point response to the issues raised in the peer review report.

**Authors' Response:** We have resolved these.

**(2) Language quality:** Please resolve all language issues in the manuscript based on the peer review report. Please be sure to have a native-English speaker edit the manuscript for grammar, sentence structure, word usage, spelling, capitalization, punctuation, format, and general readability, so that the manuscript's language will meet our direct publishing needs.

**Authors' Response:** We have resolved it (57903-Non-Native Speakers of English Editing Certificate).

**(3) Special requirements for figures:** Figures must be presented in the order that they appear in the main text of the manuscript (numbered as 1, 2, 3, *etc*.). The requirements

for the figures and figure legends include: (A) All submitted figures, including the text contained within the figures, **must be editable**. Please provide the text in your figure(s) in text boxes; (B) For line drawings that were automatically generated with software, please provide the labels/values of the ordinate and abscissa in text boxes; (C) Please prepare and arrange the figures using PowerPoint to ensure that all graphs or text portions can be reprocessed by the editor; and (D) In consideration of color-blind readers, please avoid using red and green for contrast in vector graphics or images.

**Authors' Response:** We have added Table 1 and Figure 1.

**(4) Special requirements for tables:** Tables must be presented in the order that they appear in the main text of the manuscript (numbered as 1, 2, 3, *etc*.). Please verify that the tables are referred to in the text by their respective Roman numerals and that the numbering order is correct, and format the tables. Please verify that there are no missing or multiple spaces in the text and tables, *e.g.* before or after parentheses, between words, or before or after symbols like +, ×, ±, <, >, ≥, and ≤. Please verify that the special words or letters in the text and tables are correct, *e.g.* *P* (uppercase), *n* (lowercase), *via*, *vs* (lowercase, no punctuation), *in vivo*, *in vitro*, and *et al* (no punctuation) are italicized.

**Authors' Response:** We have added Table 1.

**(5) Special requirements for references:** Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout. NOTE: The PMID is required, and NOT the PMCID; the PMID number can be found at https://pubmed.ncbi.nlm.nih.gov. (Please begin with PMID:) The DOI number can be found at http://www.crossref.org/SimpleTextQuery/. (Please begin with DOI: 10.**).

Please verify that the references are cited by Arabic numerals in square brackets and superscripted in the text, and that the numbering order is correct. There should be no space between the bracket and the preceding word or the following punctuation. When references in the text and tables are cited with author name(s), it is necessary to manually verify that the name(s) is consistent with the first author's surname in the corresponding reference list.

**Authors' Response:** We have revised the references. Reference 34, 47 and 126 do not have DOI number.

**(6) Special requirements for article highlights:** If your manuscript is an original study (basic study or clinical study), meta-analysis, systemic review, the "article highlights" section should be provided. Detailed writing requirements for "article highlights" can be found in the Guidelines and Requirements for Manuscript Revision.

**Authors' Response:** Not applicable.

**(7) Ethical documents:** Please double check the accuracy of all ethical documents and verify the completeness of the documents according to the type of manuscript.

**Authors' Response:** Not applicable.

**(8) Approved grant application form(s) or funding agency copy of any approval document(s):** If your manuscript has supportive foundations, the approved grant application form(s) or funding agency copy of any approval document(s) must be provided.

**Authors' Response:** We have changed the approved grant application form (57903-Approved Grant Application Form) in the new manuscript on PAGE 1:

Supported by National Natural Science Foundation of China, No. 81772563

Step 3: Manuscript revision deadline

**Authors' Response:** We have applied for extension and revised on 2020/09/07.

## Step 4: Verify the accuracy of general information for your manuscript

**Name of journal:** World Journal of Stem Cells

**Manuscript NO.:** 57903

**Column:** Review

**Title:** Advance in metabolism and target therapy in breast cancer stem cells

**Authors:** Xu Gao and Qiong-Zhu Dong

**Corresponding author:** Xu Gao, MD, Doctor, YiWu Maternity and Children Hospita, YiWu Maternity and Children Hospita, YiWu Maternity and Children Hospital, No.C-100 Xinke Road, Yiwu 322000, Jinhua, Zhejiang, China., Yiwu 322000, Zhejiang, China. gao.xu.199@gmail.com

**Received date:** 2020-07-01

**First decision:** 2020-07-30

**Authors' Response:** We have revised these in the new manuscript.

## Step 5: Peer-review report(s)

The authors must resolve all issues in the manuscript based on peer-review report(s) and make a point-to point response to the issues raised in the peer-review report(s) which listed below:

**Reviewer #1:**

Scientific Quality: Grade D (Fair)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: In this review article, the authors attempted to summarize recent advances in breast cancer stem cell's metabolic features and targeted therapies. Although a summary of such new information and recent progresses could be helpful to the research society, this manuscript would be improved if the authors can

address the following concerns or comments. The authors listed a number of potential cancer stem cell (CSC) markers, including CD44/CD24, CD133 and ALDH1. (1) However, it is unclear which marker(s) are specific for breast CSCs. What are the advantage and limitations of using these markers for breast CSC analysis? It is advisable to include a table to summarize the type/name of markers, biological functions, methods of analysis, % positive cells in tumor tissues, and source of references. (2) Lower levels of reactive oxygen species (ROS) have been found to be associated with breast CSCs. This could be the result of the metabolic reprogramming of CSCs. The authors should include some of the most recent studies on energy and fatty acid metabolism in CSCs. Some of references cited are a little out of date and not a refection of the recent advances in the field. (3) It would be helpful if the authors could provide one or two schematic diagrams/figures summarizing the mechanisms of drug resistance in BCSCs and therapeutic approaches to addressing the CSCs-associated clinical problems such as metastasis and tumor recurrence. (4) Some minor issues of English spelling and/or grammars need to be corrected. for examples, "EpCAM has also recently been identified as a marker for BCSCs and in involved in the promotion of bone metastases" on page 4, and "Cancer cell metabolism is characterized by dysregulated glucose metabolism, fatty acid synthesis and glutaminolysisin" on page 7.

**Authors' Response:** **We thank the reviewer for this comment.**

(1) We have added the content and Table 1 in the new manuscript on PAGE 5 and 7：

Importantly, the subpopulation of breast cancer cells with CD44+/CD24− phenotype and high ALDH enzymatic activity has become the "gold standard" signature for BCSCs, because 20 positive cells have tumorigenicity[41-43].

Although many BCSCs markers have been found by flow cytometry (summarized in Table 1), more differentiated BCSCs markers usually alter

according to different subtypes breast cancer cells, histological stage and internal heterogeneity[60]. Moreover, there is no universal marker that is specific for identification of BCSCs. These BCSCs markers mentioned above are also used to isolate other types of CSCs. In patients with breast cancer accompanied by other primary tumors, the use of BCSCs markers are limited. Therefore, further studies are still needed to determine the function and mechanism of BCSCs markers in breast cancer cells.

**Table 1 Breast cancer stem cell markers**

| Markers | Expression | Function | Ref. |
|---|---|---|---|
| CD44 | High | Adhesion, intracellular signaling, cellular proliferation, angiogenesis, differentiation, migration and invasive | Senbanjo *et al*[123], 2017 |
| CD24 | Low | Cell metastasis and proliferation | Jaggupilli *et al*[124], 2012 |
| ALDH1 | High | Cellular proliferation, differentiation, stemness and self-protection | Tomita *et al*[40], 2016 |
| CD133 | High | Cellular differentiation | Glumac *et al*[52], 2018 |
| CD49f | High | Tumor initiation, metastasis and chemoresistance | Meyer *et al*[125], 2010 |
| CD61 | High | Tumor initiation and metastasis | Zhu *et al*[126], 2019 |
| CD90 | High | Cell metastasis | Sauzay *et al*[127], 2019 |

| | | | |
|---|---|---|---|
| EpCAM | High | Cellular proliferation, migration and invasion | Sauzay *et al*[128], 2018 |

CD44: Cluster of differentiation 44; CD24: Cluster of differentiation 24; ALDH1: Aldehyde dehydrogenase 1; CD133: Cluster of differentiation 133; CD49f: Cluster of differentiation 49f; CD61: Cluster of differentiation 61; CD90: Cluster of differentiation 90; EpCAM: Epithelial Cell Adhesion Molecule.

(2) We have added the content about ROS and fatty acid metabolism in CSCs in the new manuscript on PAGE 8 and 9：

Although studies have shown that BCSCs is dependent on glycolysis, other studies have shown that BCSCs may also depend on mitochondrial oxidative phosphorylation (OXPHOS) metabolism[71-73]. Compared to their differentiated progeny, BCSCs are more dependent on OXPHOS[73, 74]. BCSCs produces less lactate and lower levels of reactive oxygen species (ROS) by OXPHOS[69]. Meanwhile, BCSCs have more functional mitochondria, and have higher ATP content by OXPHOS[75]. Interestingly, emerging evidence shows that BCSCs have a preference for OXPHOS metabolism in the proliferative state[69, 72, 76]. In the quiescent state, BCSCs exhibit higher glycolytic rate[10, 75]. Moreover, metabolism of BCSCs is plastic. The different metabolic patterns of BCSCs might be due to their distinctive molecular characteristics[72, 77, 78]. It was demonstrated that the metabolic switch from OXPHOS to aerobic glycolysis was essential for the maintenance of CD44+/CD24-/ EpCAM+ BCSCs in response to the decreased ROS levels[79]. It has been observed that BCSCs display two interchangeable states: quiescent mesenchymal-like (M) states and proliferative epithelial-like (E) states[80, 81]. M-BCSCs are characterized by elevated aldehyde dehydrogenase (ALDH) activity and proliferative capacity. Differently, E-BCSCs exhibit CD44+/CD24- cell surface marker expression and quiescent state[78, 82].

We have also updated recent related references.

(3) We have added the figure (Figure 1) to summarize the mechanisms of drug resistance in BCSCs and therapeutic approaches.

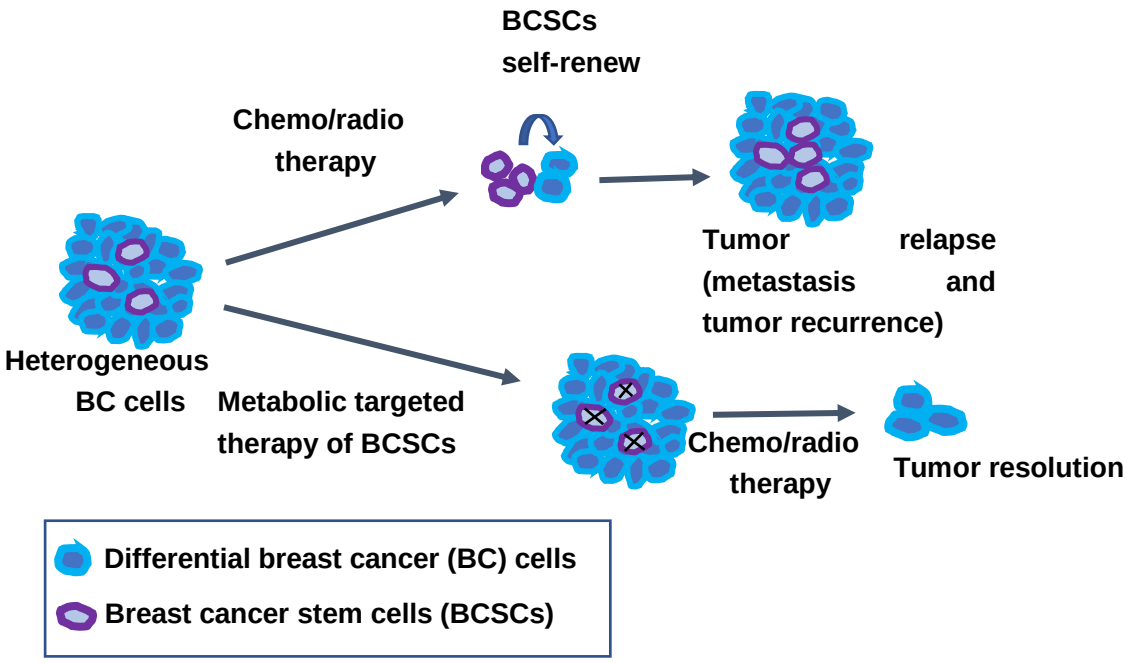


**Figure 1 Metabolic targeted therapy of BCSCs.** Breast cancer stem cells (BCSCs) have been demonstrated to contribute to tumor heterogeneity and tumor relapse (metastasis and tumor recurrence). The metabolic features of BCSCs significantly differ from non-BCSCs differentiated tumor cells. Conventional chemotherapy or radiotherapy can effectively kill breast cancer cells, thereby dramatically reducing tumor size. The remaining BCSCs can survive and enhance tumor relapse due to their ability to establish higher invasiveness and resistance to chemotherapy and radiotherapy. A combination treatment with metabolic targeted therapy of BCSCs and conventional therapy could be a more effective strategy to treat breast cancer.

(4) We have corrected it in the new manuscript on page 5-6 and 7.

Recently, EpCAM has been identified as a marker of BCSCs and participates in promoting bone metastasis by enhancing tumorigenicity.

Cancer cell metabolism is characterized by dysregulated glucose metabolism, fatty acid synthesis and glutaminolysis to provide constant support for the increased division rate[61-63].

**Reviewer #2:**

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: The authors made a job to summarize about BCSC. (1) However, since the title of this manuscript claim to analyse the CSC metabolims, I suggest to improve the data and evidences of this topic, even I saw this relevant section too short. The therapeurtic treatment to target BSCS was address well done. (2) Also I suggest to include an figure to summarize the manuscript.

**Authors' Response:** **We thank the reviewer for this comment.**

(1) We have added the content in the new manuscript on page 7-9.

Due to the small number of studies on the metabolism of CSCs, especially BCSCs, the metabolic features of CSCs remain largely unknown[65]. Nonetheless, limited studies show that the metabolic characteristics of CSCs are different from normal cells and cancer cells. Glucose is an essential nutrient for CSCs like that of cancer cells. More specifically, CSCs (including BCSCs) were more glycolytic than other differentiated cancer cells[66]. CSCs show increased glucose uptake, lactate production and ATP content compared with other differentiated cancer cells[67]. Several studies have supported that BCSCs showed enrichment of glycolytic protein (such as PKM2 and LDHA), as well as increased pyruvate kinase and lactate dehydrogenase activities[68, 69]. Meanwhile, the levels of glycolysis intermediates, such as fructose 1,6-diphospate, pyruvate, lactic acid, and ribose 5-phosphate were found to be

significantly higher in BCSCs[70]. Thus, the inhibitors of glycolysis are expected to be used to treat cancer with metabolic targeted therapy. Reports have demonstrated that inhibitors of glycolysis reduce the proliferation of BCSCs[21, 69].

Although studies have shown that BCSCs is dependent on glycolysis, other studies have shown that BCSCs may also depend on mitochondrial oxidative phosphorylation (OXPHOS) metabolism[71-73]. Compared to their differentiated progeny, BCSCs are more dependent on OXPHOS[73, 74]. BCSCs produces less lactate and lower levels of reactive oxygen species (ROS) by OXPHOS[69]. Meanwhile, BCSCs have more functional mitochondria, and have higher ATP content by OXPHOS[75]. Interestingly, emerging evidence shows that BCSCs have a preference for OXPHOS metabolism in the proliferative state[69, 72, 76]. In the quiescent state, BCSCs exhibit higher glycolytic rate[10, 75]. Moreover, metabolism of BCSCs is plastic. The different metabolic patterns of BCSCs might be due to their distinctive molecular characteristics[72, 77, 78]. It was demonstrated that the metabolic switch from OXPHOS to aerobic glycolysis was essential for the maintenance of CD44+/CD24-/ EpCAM+ BCSCs in response to the decreased ROS levels[79]. It has been observed that BCSCs display two interchangeable states: quiescent mesenchymal-like (M) states and proliferative epithelial-like (E) states[80, 81]. M-BCSCs are characterized by elevated aldehyde dehydrogenase (ALDH) activity and proliferative capacity. Differently, E-BCSCs exhibit CD44+/CD24- cell surface marker expression and quiescent state[78, 82].

Fatty acid (FA) is the conversion of various nutrients in cells into metabolic intermediates, which are essential for maintaining the structure and function of cell membranes, energy storage and signal transduction[83]. In addition to glucose metabolism, cells also generate energy by breaking down FA by fatty acid oxidation (FAO)[84]. FAO is also important for cancer cell survival and chemotherapy resistance[26]. CSCs needs to metabolize FA through FAO to generate energy to maintain survival[85]. It was reported that CD44+/CD24-

BCSCs contain high levels of lipid droplets and the number of lipid droplet correlates with the stemness of breast cancer cells. Chemical small molecule inhibitors targeting lipid metabolism directly impact the mammosphere formation of BCSCs. It was demonstrated that JAK/STAT3 signaling regulates lipid metabolism, which promotes BCSCs stemness and breast cancer chemoresistance. Inhibiting JAK/STAT3 signaling or blocking FAO can prevent BCSCs maintenance and breast cancer chemoresistance. Drug-induced inhibition of mitochondrial FAO with etomoxir impairs NADPH production and increases the levels of ROS. Importantly, etomoxir significantly inhibits tumorsphere formation of radiation-derived breast cancer stem cells (RD-BCSCs). Promyelocytic leukemia (PML) gene was enriched in triple-negative breast cancers. It was reported found that PML acted as a potent activator of PPAR signaling and fatty acid oxidation. Recent studies have demonstrated the critical role of PML in CSCs. Therefore, BCSCs from triple-negative breast cancers may depend on fatty acid oxidation. Until now, there are few studies on the metabolic characteristics of BCSCs metabolism. Thus, well-defined features of BCSCs metabolism still need to be depicted.

(2) We have added a figure (Figure 1) to summarize the manuscript.

**Step 6: Editorial Office's comments**

The author must revise the manuscript according to the Editorial Office's comments and suggestions, which listed below:

**(1) Science Editor:** 1 Scientific quality: The manuscript describes a review of the advance in metabolism and target therapy in breast cancer stem cells. The topic is within the scope of the WJSC. (1) Classification: Grade B and Grade D; (2) Summary of the Peer-Review Report: The authors summarized the recent advances in breast cancer stem cell's metabolic features and targeted therapies. However, the data and evidences about this topic should be added. And a

figure to summarize the manuscript should also be added. The questions raised by the reviewers should be answered; and (3) Format: There are no tables and figures. A total of 114 references are cited, including 56 references published in the last 3 years. There are no self-citations. 2 Language evaluation: Classification: Grade B and Grade B. The language was edited by a native English speaker. 3 Academic norms and rules: The Conflict-of-Interest Disclosure Form and Copyright License Agreement are not right. No academic misconduct was found in the CrossCheck detection and Bing search. 4 Supplementary comments: This is an invited manuscript. The study was supported by a national grant. The topic has not previously been published in the WJSC. 5 Issues raised: (1) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s); and (2) PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout. 6 Re-Review: Required. 7 Recommendation: Conditional acceptance.

**Authors' Response:** **Our previous replies have solved these problems.**

**(2) Editorial Office Director:** I have checked the comments written by the science editor. The authors need to complete the Conflict-of-Interest Disclosure Form and provide the Copyright License Agreement signed by all authors. The authors need to add some figures or tables.

**Authors' Response:** **Our previous replies have solved these problems.**

**(3) Company Editor-in-Chief:** I have reviewed the Peer-Review Report, the full text of the manuscript and the relevant ethics documents, all of which have met the basic publishing requirements, and the manuscript is conditionally

accepted with major revisions. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report and the Criteria for Manuscript Revision by Authors. Re-Review: Required.

**Authors' Response:** **We have revised these.**

**Step 7: Revise the manuscript**

Please update your manuscript according to the Guidelines and Requirements for Manuscript Revision and the Format for Manuscript Revision for your specific manuscript type: 'Review'. Please visit https://www.wjgnet.com/bpg/GerInfo/291 for the article type-specific guidelines and formatting examples. We only accept the manuscript in MS Word format, and the manuscript in other formats will be rejected.

**Authors' Response:** **We have revised these.**

**Step 8: Submit the revised manuscript and all related documents**

In order to be able to edit your manuscript in time and with high quality, and make it finally be accepted and officially published, please submit your revised manuscript online based on the revised version in MS Word format, by following the '**Steps for Submitting Revised Manuscript**'. The steps for submitting your revised manuscript include: (1) Author Information; (2) Manuscript Information; (3) Abstract, Main Body, and Acknowledgements; (4) References; (5) Footnotes and Figure Legends; (6) Automatically Generate Full Text Files; and (7) Upload the Revision Files. There are 'Guidelines for Writing' for each step of submitting your revised manuscript. When steps 1-5 are completed, the full text file will be automatically generated and stored in the Uploaded Files, and you will not need to upload it manually. **The figures and tables should be uploaded separately to the Uploaded Files.**

For step 4 (References), please be sure to edit the references by Auto-Analyser to ensure the correctness of all reference information. Please start to test the references by clicking on the "Edit References by Auto-Analyser" button on the page. The specific steps are: (1) Copy all references to the Auto-Analyser. The reference list should begin with Arabic number "1", and please do not use brackets for the references numbers; (2) click on the "Check If There Are Any Duplicate References" button to detect potential duplicate references in the reference list; (3) click on the "Edit References by Auto-Analyzer" button to automatically edit and standardize the format of references with a PMID number; (4) verify the list of references that have undergone automatic edition and standardization in the "Proofreading of the References" and "Auto-Edited References Preview" pages under the "Result of Analyze"; and (5) click on the "Confirm" button at the bottom of the page and the system will automatically replace and update the list of references online.

For all required accompanying documents (listed below), you can begin the uploading process *via* the F6Publishing system.

(1) 57903-Manuscript File **YES**

(2) 57903-Answering Reviewers **YES**

(3) 57903-Audio Core Tip **YES**

(4) 57903-Conflict-of-Interest Disclosure Form **YES**

(5) 57903-Copyright License Agreement **YES**

(6) 57903-Approved Grant Application Form(s) or Funding Agency Copy of any Approval Document(s) **YES**

(7) 57903-Non-Native Speakers of English Editing Certificate **YES**

(8) 57903-Video **Not applicable**

(9) 57903-Image File **YES**

(10) 57903-Table File **YES**

(11) 57903-Supplementary Material **Not applicable**

**Authors' Response:** **We have revised these.**