

Reviewer Code: 03478635

This review article contains new visions in which biliary tract cancer has cancer stem cells. Please clarify more in detail and discuss how interleukin (IL)-6 / JAK / STAT cascade is involved in CSC signaling in terms of cross-talk with NOTCH, Wnt or Hh signaling in Relevance of stemness pathways in biliary tract cancer stem cell section.

We thank the reviewer for his or her positive comment on our manuscript.

In the current manuscript we have focused on studies using patient material and to our knowledge, not paper currently exists that clearly describes a cross-talk between the mentioned signaling pathways.

However, we have expanded the information regarding IL-6 and inflammation and have added a sentence in the 'Conclusion' that addresses your suggestion.

Please check references and abbreviations carefully again

We have checked references and abbreviations and updated the manuscript accordingly.

Reviewer Code: 03370303

This review is very well written, intensively illustrating the characteristics and impact of biliary tract cancer stem cells from both scientific and clinical point of view. It will make a great contribution to an advanced understanding of the pathophysiology of biliary tract cancers.

[We thank the reviewer for his or her positive comment on our manuscript.](#)

Although the quality of English is satisfactorily high throughout the main text, there are several points to be modified in Abstract and Core tip. Before publication in World Journal of Gastroenterology, they should appropriately be addressed.

Grammatical/terminological concerns:

1. In the 6th line in Abstract, the phrase "...due to their suspected contributions..." sounds rather peculiar since the word "suspected" is generally used with a negative (bad) connotation. It would be better to rewrite the phrase as "...due to their potential contributions...", for example.

[Changed as suggested.](#)

2. In the 1st line in Core tip, the phrase "researchers could demonstrate ..." should be written in the past tense without using the word "could", because this auxiliary verb even implies "possibility" or "conjecture". It would be better to rewrite the phrase as "researchers successfully demonstrated...", for example.

[Changed as suggested.](#)

3. In the 6th line in Core tip, it would be better to rewrite the phrase "Following these results..." as "Based on these results...".

[Changed as suggested.](#)

4. In the 6th line in Core tip, it would be better to rewrite the phrase "the existence of cancer stem cells in biliary tract is likely..." as "the existence of cancer stem cells in biliary tract is well founded" or "the existence of cancer stem cells in biliary tract is valid". The word "likely" rather lacks scientific tone.

[Changed as suggested.](#)

5. In the last sentence in Introduction, the word "BTC" should be written together with its non-abbreviated form since this is the first appearance of this term in the manuscript.

[Changed as suggested. We have revised the manuscript for similar corrections.](#)

6. In line 8 in page 5, the phrase "very few cells are seeded..." should be rewritten with concreteness. For example, it should be written as "very few cells (e.g. 100 cells /25 cm²) are seeded...."

[As suggested, an approximate range of cell densities \(cell line-dependent\) has been added in this sentence.](#)

Reviewer Code: 02566177

This review focuses on new concepts and recent discoveries on cancer stem cells related to the biliary tract. These concepts are relatively new and very important to understand tumor biology and natural history of these cancers that still have a dismal prognosis overall. The review is reasonably well written (a review by an English first language expert is warranted).

We thank the reviewer for his or her positive comment on our manuscript.

My only point: I would probably underscore in more detail the role of inflammation and particularly chronic inflammation and its relations to cancer stem cells establishment and natural history of the disease

In the revised manuscript, the paragraph regarding the suggested role of inflammation and biliary cancer stem cells has been expanded. Also in response to another reviewer's comment, at two other positions in the revised manuscript (chapters "Relevance of Stemness Pathways in Biliary Tract Cancer Stem Cells" and "Conclusion") the specific role of IL-6 signaling in the pro-inflammatory environment has been detailed.

In our opinion underlining the role of inflammation in cancer stem cells in general or its relation to the natural course of the disease (besides its role for CSC signaling) is beyond the scope of this manuscript.

Reviewer Code: 03471529

I think that the manuscript is very well written, intensively reviewing biliary tract cancer stem cells, and insighting the pathophysiology of biliary tract cancers.

[We thank the reviewer for his or her positive comment on our manuscript.](#)

However, it is conceivable that the authors should comment as follows in the section of Cancer Stem Cell Markers in Biliary Tract Cancer – an Overview. ESC-genes such as POU5F1, SOX2 and NANOG are essential for their self-renewal ability and pluripotency. Therefore, CSCs should express these genes at a level equivalent to those of ESCs for such function. From a functional point of view, POU5F1, SOX2 and NANOG were not always the marker genes for CSCs and drug resistance. In addition, lower level of the expression was not always functional for the self-renewal ability and pluripotency.

[We thank the reviewer for commenting on this aspect of cancer stem cell signaling. We fully agree that isolated expression of the mentioned ESC-genes is necessarily neither a stand-alone marker for identifying cancer stemness nor conveying drug resistance mechanisms. Due to the limited number of previously published studies on the specific role \(or relevance\) of these markers for BTC or biliary tract cancer stem cells, we focused the corresponding section in the manuscript on the sparse data available and believe that it adequately represents the currently published data in this regard.](#)