Jean-Pierre Aubert Research Center

Neurosciences & Cancer **UMR-S 1172**

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Object: Revised manuscript NO: 47658

Dear Editor,

We are pleased to submit a revised version of our manuscript NO: 47658 entitled "Colon cancer stemness as a reversible epigenetic state: Implications for anticancer therapies".

We have perfected the manuscript according to peer-reviewer's comments. All modifications in the text appear in red characters. Specific point-to-point replies are listed below:

Reviewer #1

In discussion site, the authors should describe whether or not this story is limited in colon cancer.
This question has been addressed before the conclusion paragraph (p15).

Reviewer #2

The numbering of the main and secondary sections is confusing.
The numbering of the sections has been corrected.

Reviewer #3

There is no any information that just an erasure of epigenetics marks of differentiated cells will reverse cells as it is mentioned by Authors in the 1st section. Cited literature refers to incompletely reprogrammed by transcription factors somatic cells (13) or even unknown manuscript (14).
We have modified the aforementioned paragraph (p5) and corrected the reference #14.

Stem cells are found in different tissues during ontogeny and their function is tissue maintaining and regeneration by differentiation program. Thus only epigenetic mechanisms are involved in functional changes of stem cells. Tumor cell evolution from normal somatic state towards undifferentiated phenotype is based on the genetic changes and clonal selection therefore epigenetic modifiers may have opposite effects on tumor cells sensitivity to different drugs due to their unknown genetics within the tumor tissue.
We agree that unlike normal differentiation processes where only epigenetic changes are observed, carcinogenesis involves both genetic and epigenetic changes and that genetic alteration can affect cell sensitivity to epidrugs. We added this crucial consideration to the manuscript (p5).

Surprisingly that Authors describe it as a "rapid phenotype switches, is probably mediated by epigenetic mechanisms that are reversible in nature, rather than by permanent genetic mutations 9". Ref 9 clearly demonstrates that appearance of some secondary markers of tumor cells (ALDH1, proteasome activity but not functional properties of each tumor "stem" cell) are due to significant genetic changes (radiation or polyploidy) however

unrelated to any epigenetic events.
Our statement has been turned into a hypothesis in the manuscript and a reference has been added to support the epigenetic nature of “naturally”-acquired (without treatment with radiotherapy or chemotherapy) stemness properties in vitro (p5).

Generally, it is accepted that tumors are composed of subpopulations of cells that can be distinguished on the basis of a variety of genetic features that affect their phenotype (SNVs, indels, somatic CNAs, chromosome variants). Genetic intratumor heterogeneity has been documented across most cancers (McGranahan, N. & Swanton 2015 Turajlik et al 2019) and acts as a substrate for clonal evolution. Evolution is not programmable and it is unclear whether all cells within the tumor have the same so called “program” or various.
To my knowledge there is no any information about genetic homogeneity of so called cancer stem cell (or cancer/tumor initiating cell) while tumor genetic heterogeneity is well established. I would advise to Authors use terms more accurately according to their real meaning or to provide more explanations.
We agree with the global comments of reviewer 3 that genetic alterations leading to tumor heterogeneity have been overlooked in the first version of our manuscript. Although these considerations had been suggested (p.12): “initiating re-differentiation in cancer stem cells remains a challenge dependent on the characteristics of each tumor type and with their specific genetic alterations”, we substantially modified several statements throughout the manuscript as we realized that some of our conclusions led to some understandable critics (p4, 5, 10, 11). For instance, we systematically considered “epigenetic signatures” as plural since genetically diverse cancer stem cells may harbor different epigenetic profiles. Also, we have distinguished more clearly pre-existing cancer stem cells (cells of origin of a tumor) from induced cancer stem cells (through clonal evolution, administrated therapy or epigenetic changes) that probably harbor very different mutation profiles (Figure 1 and 3 and p5). Some references have been added accordingly (p4 and 10). Despite genetic heterogeneity among cancer stem cells due to stochastic events, our hypothesis is that cancer stem cell marker silencing/overexpression could be triggered by comparable epigenetic mechanisms and therefore could be targetable. We hope the modifications we made in the manuscript make our hypothesis clearer.

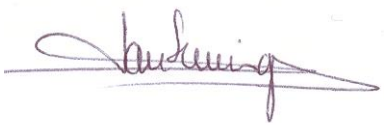
I would also recommend to reduce the number of Tables. Actually in the manuscript Authors refers only to 3 of them (1, 5, 8) in support of their statements.
Tables 1, 2 and 3 have been combined. All other tables have been better cited in the manuscript to support our statements.

Additionally, unnecessary abbreviations have been removed from the text and from the figure/table titles. The abbreviations in the figure and tables have been added to the legends. All the necessary corrections have been made in the reference section.

We hope that the modifications we made in this revised manuscript will fulfill your expectations and that you will find this version of interest for publication in your journal.

I stay at your disposal for any supplemental information.

Yours sincerely,



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