

Oncofetal reprogramming drives phenotypic plasticity in WNT-dependent colorectal cancer

Corresponding Author: Dr Ernesto Guccione

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Genetics.

Version 0:

Decision Letter:

30th Aug 2024

Dear Ernesto,

Your Article, "Oncofetal reprogramming fuels phenotypic plasticity in WNT-driven colorectal cancer" has now been seen by 2 referees. Reviewer #2 is fully satisfied with the revised manuscript. However, Reviewer #1 has a remaining concern regarding the simultaneous need of the OnF and SC states for complete drug response. We are interested in the possibility of publishing your study in Nature Genetics, but would like to consider your response to this concern in the form of a revised manuscript before we make a final decision on publication. Therefore, we suggest you to make Reviewer #2's required textual changes. Please do not hesitate to get in touch if you would like to discuss these issues further.

We therefore invite you to revise your manuscript taking into account all reviewer and editor comments. Please highlight all changes in the manuscript text file. At this stage we will need you to upload a copy of the manuscript in MS Word .docx or similar editable format.

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It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review.

A revised checklist is essential for re-review of the paper.

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We hope to receive your revised manuscript within four to eight weeks. If you cannot send it within this time, please let us know.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further.

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We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Sincerely,
Chiara

Chiara Anania, PhD
Associate Editor
Nature Genetics
<https://orcid.org/0000-0003-1549-4157>

Referee expertise:

Referee #1:

Referee #2:

Referee #3:

Reviewers' Comments:

Reviewer #1:

Remarks to the Author:

My concerns raised in the previous review have been satisfactorily addressed.

Regarding the terminology adjustment, I agree with the authors that there is a transition of LGR5+ stem cell state to a hybrid state, but from the data demonstrated in Fig.4c-d, it shows clearly that both OnF and stem cell state alone were downregulated after FOLFIRI treatment (OnF: 7.04% to 5.67%; SC: 24.5% to 12%), while the hybrid state was remarkably activated from 9.66% to 44.4%. This indicates that it is the hybrid state, but not the pure OnF state, that is induced to drive drug tolerance. This is a very important finding as their subsequent in vivo data confirms that depletion of both states simultaneously (but not alone) is required to yield complete response to FOLFIRI. I'd therefore argue that the author needs to improve the clarity of this key finding in the abstract and the text. For instance, in the revised abstract, it states "...targeting the OnF program in combination with the current standard of care is pivotal for achieving effective and durable CRC treatment.". This is inaccurate since targeting either OnF or SC state alone yield the same partial drug response. Only simultaneous targeting of both OnF and SC state shows complete drug response (Fig.4I). Therefore, the accurate statement would be "simultaneous targeting of both the OnF and the canonical stem cell program in combination with...." to reflect their key findings.

Reviewer #2:

Remarks to the Author:

This revised version adequately addresses our previous comments, we therefore support its publication and congratulate the authors for their work.

Michael Tyler and Itay Tirosh

Version 1:

Decision Letter:

Our ref: NG-A66151R

23rd Sep 2024

Dear Ernesto,

thank you for submitting your revised manuscript "Oncofetal reprogramming fuels phenotypic plasticity in WNT-driven colorectal cancer" (NG-A66151R). We find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Genetics, pending minor revisions to satisfy to comply with our editorial and formatting guidelines.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements soon. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Genetics and please do not hesitate to contact me if you have any questions.

Congratulations!

Sincerely,
Chiara

Chiara Anania, PhD
Associate Editor
Nature Genetics
<https://orcid.org/0000-0003-1549-4157>

Version 2:

Decision Letter:

In reply please quote: NG-A66151R1 Guccione

11th Dec 2024

Dear Dr. Guccione,

I am delighted to say that your manuscript "Oncofetal reprogramming drives phenotypic plasticity in WNT-dependent colorectal cancer" has been accepted for publication in an upcoming issue of Nature Genetics.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Genetics style. Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required.

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Sincerely,
Chiara

Chiara Anania, PhD
Associate Editor
Nature Genetics
<https://orcid.org/0000-0003-1549-4157>

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Reviewer #1:

Remarks to the Author:

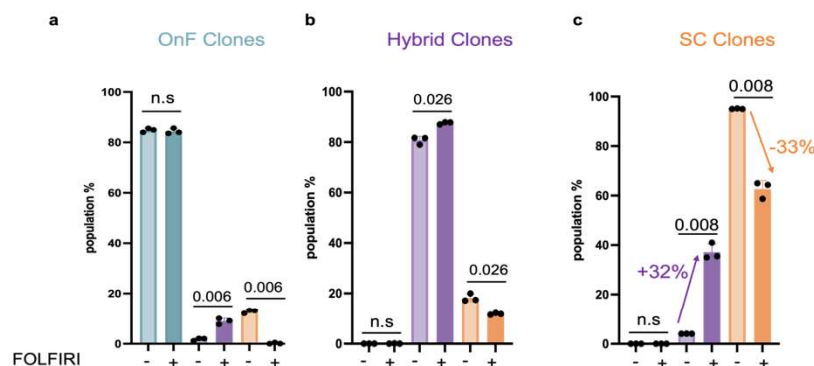
My concerns raised in the previous review have been satisfactorily addressed.

Regarding the terminology adjustment, I agree with the authors that there is a transition of LGR5⁺ stem cell state to a hybrid state, but from the data demonstrated in Fig.4c-d, it shows clearly that both OnF and stem cell state alone were downregulated after FOLFIRI treatment (OnF: 7.04% to 5.67%; SC: 24.5% to 12%), while the hybrid state was remarkably activated from 9.66% to 44.4%. This indicates that it is the hybrid state, but not the pure OnF state, that is induced to drive drug tolerance. This is a very important finding as their subsequent in vivo data confirms that depletion of both states simultaneously (but not alone) is required to yield complete response to FOLFIRI. I'd therefore argue that the author needs to improve the clarity of this key finding in the abstract and the text. For instance, in the revised abstract, it states "...targeting the OnF program in combination with the current standard of care is pivotal for achieving effective and durable CRC treatment.". This is inaccurate since targeting either OnF or SC state alone yield the same partial drug response. Only simultaneous targeting of both OnF and SC state shows complete drug response (Fig.4I). Therefore, the accurate statement would be "simultaneous targeting of both the OnF and the canonical stem cell program in combination with...." to reflect their key findings.

We are pleased that all reviewers were satisfied with the revised manuscript.

In response to Reviewer #1, we have adjusted the terminology in the text to clarify the points raised. We would like to bring to this reviewer's attention that the marginal fluctuation in the pure OnF state OnF: 7.04% to 5.67% is not statistically significant ($p = 0.096$; Fig. 4d).

We acknowledge that it is perhaps challenging to draw definitive conclusions from the concurrent analyses of all 3 cell states (Fig. 4c-d). To address this, we conducted a more detailed assessment of individually sorted clones, which are predominantly in either an OnF, Stem, or Hybrid state, to evaluate their response to FOLFIRI. Below, we present a more detailed breakdown of the changes observed in each state across the various clones in response to FOLFIRI (panels a-c). These data provide compelling evidence that:



1. The proportion of cells in the pure OnF state remains largely unchanged (panel a below - break downs of original Ext. Data Fig. 4j)). Notably, there's a significant increase in hybrid cells, which is better understood in light of data presented in panel c, discussed point 2 below.

The persistence of cells with an active OnF program—encompassing both pure OnF and Hybrid cells—demonstrates their inherent resistance to FOLFIRI. As requested by this reviewer, we have revised the terminology in the text to clarify that cells "with an active OnF program" includes both the pure OnF and hybrid states.

2. Conversely, we observed a specific and substantial reduction of cells in the pure LGR5⁺ SC state (-33%), which coincided with a corresponding increase in the hybrid state (+32%) (most

evident in **Panel c**). This demonstrates that cells in the pure **LGR5⁺ SC** state are sensitive to FOLFIRI, and it suggests an activation of the of the OnF program in the surviving SCs.

In line with this reviewer's request, we have modified the abstract to clarify 1) that drug-tolerant states (pure OnF and Hybrid) are characterized by an active OnF program, and 2) that the pure/canonical LGR5⁺ SC state is sensitive to FOLFIRI.

This provides a rationale for **combining FOLFIRI, which targets the pure LGR5⁺ SC state**, with modalities/**therapies that inhibit the OnF program** (such as DT or inhibitors of YAP and AP-1) **to achieve a more effective and durable treatment for CRC, as demonstrated in our Fig. 4I-q**.

In brief, the experiments in **Fig. 4I-q** provide compelling evidence that targeting cells with an active OnF program -pure OnF and Hybrid- in combination with FOLFIRI is an effective strategy. Further targeting the pure LGR5⁺ SC state program, as suggested, is redundant with the use FOLFIRI, which is sufficient to deplete this population (**panel c**). Mechanistically, FOLFIRI depletes canonical LGR5⁺ SCs, but not entirely; those who survive this treatment have activated the OnF program, entering a hybrid state. Therefore, successful targeting of this program or its drivers (YAP/AP-1) enhances the effectiveness of FOLFIRI by eliminating these hybrid cells as well as the pure OnF cells.

Please provide a point-by-point response to the following requests:

-As previously requested, please include an **Ethics** section as a first paragraph of the Methods and a **Code Availability Statement**.

-As previously mentioned, even if your study doesn't include the use of custom codes, you should please provide a Code Availability Statement clearly stating that you did not use custom codes. **The reader should be directed to details of software packages in the appropriate vignette within the Methods.** However, we strongly recommend that **all** code used should be deposited in a DOI-minting repository such as Zenodo, Gigantum or Code Ocean and the link cited in the Methods-only reference list. Authors are encouraged to manage subsequent code versions and to use a license approved by the open source initiative. Additional details can be found here: <https://www.nature.com/nature-portfolio/editorial-policies/reporting-standards#availability-of-computer-code> and <https://www.nature.com/articles/s41588-023-01411-0>.

- Please check and confirm that full version numbers are provided for all software packages.

A paragraph providing the ethics statement has been added at the beginning of the Methods section. A Code Availability Statement has also been included, and the details of the software packages and relevant citations have been verified.

-Main Fig. 1 d-l: scatter plots need color scale?

These panels do not require a color scale, as the colors represent different samples or conditions. Labels and a legend have been provided for clarity.

-Main Fig. 2a: color scale needs label?

Color scale is now labeled.