

Microbial metabolites control self-renewal and precancerous progression of human cervical stem cells

Corresponding Author: Professor Youngtae Jeong

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

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

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1. In figure 4m-o, the authors reconstructed the differentiation trajectory using pseudotime analysis, it would be better to demonstrate the molecular signatures (pathways of TFs) that activated or inactivated along the differentiation trajectory
2. In figure 8, the authors established a long-term, three-dimensional culture of human cervical precancerous organoids, so are there any molecular or cellular changes after long-term culture, compared with those of the primary culture, or short time culture?

Reviewer #3

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Authors have addressed all my concerns.

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The reviewer questions the application of organoid formation assay in identifying stem cells and why progenitors and differentiated subpopulations still form a few organoids. However, it is well-known that progenitors still retain limited self-renewal capability and multi-lineage differentiation potential. For example, multipotent progenitors (MPPs) in the hematopoietic system still self-renew, with limited ability, and can differentiate into every hematopoietic lineage. Likewise, progenitors and differentiated cells could form a few organoids and sometimes dedifferentiate into stem cells in many other systems, including intestinal and tracheal systems.

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Therefore, the fact that progenitors can dedifferentiate and generate organoids cannot weaken the proof showing stem cells' identity and self-renewal capability.

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We appreciate the reviewer's careful comment. By performing the network analysis, we aimed to identify the key intercellular regulatory pathways in three steps:

- i) intercellular interactions between every cell in the ectocervix (CCL),
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We provide comprehensive data from different aspects regarding the role of the PI3K-AKT signaling

pathway in human cervical stem cells.

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We thank the reviewer for this comment. We further analyzed our scRNA-seq dataset. In the Supplementary Fig. 6a and 6b, we identified genetic signatures differentially enriched between basal cells and suprabasal cells by performing the gene set enrichment analysis. We projected the top upregulated and downregulated pathways of “Epithelial mesenchymal transition” and “Keratinization” along the differentiation trajectory. Since these gene sets are first mentioned in the Supplementary Fig. 6a and 6b, we included these data in the Supplementary Fig. 6d, instead of Fig. 4.

2. In figure 8, the authors established a long-term, three-dimensional cultures of human cervical precancerous organoids, so is there any molecular or cellular changes after long-term culture, compared with those of the primary culture, or short time culture?

We understand the reviewer's concern since a long-term culture could induce genetic, molecular, or cellular changes in the organoids or cells, which could potentially harm the representativeness of organoids for the original tissues. Since we were well aware of this point from the beginning, we used precancerous organoids within passage 3 in our experiments. We described the following sentence in the result section in lines 525-527 and 531 marked in red, “Since long-term culture and passaging can induce genetic and molecular changes in organoids compared to their original biopsy sample, we used organoids within the third passage for the subsequent experiments”.