



## Autophagy repurposes cells during paligenosis

Zhi-Feng Miao<sup>a,b</sup>, Charles J. Cho<sup>a</sup>, Zhen-Ning Wang <sup>b</sup>, and Jason C. Mills <sup>a,c,d</sup>

<sup>a</sup>Division of Gastroenterology, Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA; <sup>b</sup>Department of Surgical Oncology and General Surgery, Key Laboratory of Precision Diagnosis and Treatment of Gastrointestinal Tumors, First Hospital of China Medical University, Shenyang, Liaoning Province, China; <sup>c</sup>Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO, USA; <sup>d</sup>Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA

### ABSTRACT

Differentiated cells have evolved paligenosis, a conserved program to return to a stem or progenitor state and reenter the cell cycle to fuel tissue repair. Paligenosis comprises three sequential stages: 1) quenching of MTORC1 activity with induction of massive macroautophagy/autophagy that remodels differentiated cell architecture; 2) induced expression of progenitor/repair-associated genes; 3) MTORC1 reactivation with cell cycle reentry. Here, we summarize work showing that evolutionarily conserved genes – *Ddit4* and *Ifrd1* – are critical regulators of paligenosis. DDIT4 suppresses MTORC1 function to induce lysosomes and autophagosomes in paligenosis stage 1. As DDIT4 decreases during paligenosis, TRP53 continues MTORC1 suppression until cells are licensed to reenter the cell cycle by IFRD1 suppression of TRP53. Cells with DNA damage maintain TRP53 until either the damage is repaired, or they undergo apoptosis. The concept of paligenosis and identification of paligenosis-dedicated genes may provide new angles to harness tissue regeneration and specifically target tumor cells.

### ARTICLE HISTORY

Received 18 November 2020  
Revised 23 November 2020  
Accepted 24 November 2020





### KEYWORDS

Acinar-ductal metaplasia (ADM); dedifferentiation; progenitor cell; regeneration; spasmodic polypeptide-expressing metaplasia (SPEM); tumorigenesis

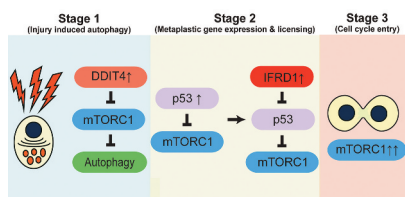
In a multicellular organism, the vast majority of cells are specialists, differentiating to perform specific physiological functions such as secretion. The only cells in an adult organism that divide routinely are dedicated stem cells in a limited number of tissues that face daily stress. For example, the mammalian gastrointestinal tract is lined by cells that are constantly sloughed and replenished by stem cells located deeper within the epithelium. However, most organs do not face routine stress or have cells that are programmed to be sloughed, and they do not have dedicated stem cells. Even organs with dedicated stem cells can face tissue damage that is more severe than can be quickly repaired by dedicated stem cells. However, differentiated cells have elaborate architecture that they develop to perform their specific functions, and have a high threshold for cell cycle entry. Accordingly, they have evolved a conserved program that carefully orchestrates their reprogramming to return to a stem or progenitor state to fuel tissue repair. This conserved program differentiated cells execute to reenter the cell cycle is called paligenosis (Figure 1). The three sequential stages of paligenosis are: 1) quenching of MTORC1 activity with induction of massive lysosome biogenesis and macroautophagy that remodels differentiated cell architecture; 2) induced expression of progenitor cell genes; 3) MTORC1 reactivation with cell cycle reentry. In a recently published manuscript, we pursued the hypothesis that because cells' capability to undergo paligenosis is conserved across tissues and species, paligenosis – like apoptosis – should be regulated by specific, dedicated genes [1].

Our search for dedicated paligenosis genes started with a hypothesis: paligenosis genes would be: 1) highly conserved across tissues and species; 2) dispensable for normal development and constitutive stem cell activity; 3) induced by injury that causes differentiated cells to reenter the cell cycle. We began our search based on our understanding of the molecular events governing the key stages of paligenosis. In the first stage, cells undergo a wholesale induction of autophagosomes and lysosomes such that a substantial portion of the cytoplasm becomes involved in this autodegradation. In our principal mouse model systems in the mouse stomach and pancreas where we observe the exocrine secretory cells undergoing paligenosis, paligenotic cells have abundant autodegradation of: endoplasmic reticulum (reticulophagy), ribosomes (ribophagy), and zymogenic secretory granules (zymophagy). After paligenotic cells turn over a large portion of their differentiated cell architecture and then induce progenitor genes, they reenter the cell cycle. Their transition into G<sub>1</sub>-S phase of mitosis requires MTORC1, as rapamycin blocks the transitions, leaving paligenotic cells in a progenitor-like – but non-proliferative – state.

Using bioinformatic screens, we identified two genes and described their roles in governing paligenosis in a recently published manuscript: *Ddit4* and *Ifrd1*. Already well-known as an evolutionarily conserved gene that suppresses MTORC1 function by inducing the TSC1-TSC2 complex, DDIT4 regulates stage 1 of paligenosis. In the stomach body, *Ddit4* is expressed specifically in chief cells, the digestive-enzyme-

**CONTACT** Jason C. Mills  [jmills@wustl.edu](mailto:jmills@wustl.edu)  Division of Gastroenterology, Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA; Zhen-Ning Wang  [josieon826@sina.cn](mailto:josieon826@sina.cn)  Department of Surgical Oncology and General Surgery, Key Laboratory of Precision Diagnosis and Treatment of Gastrointestinal Tumors, First Hospital of China Medical University, Shenyang, Liaoning Province 110001, China

\*Contributed equally.



**Figure 1.** Differentiated cells have evolved a conserved program to return to a stem or progenitor state to fuel tissue repair, also known as paligenesis, which comprises three sequential stages: 1) MTORC1 quenching with macroautophagy induction; 2) induced progenitor cell gene expression; 3) licensed cells reactivate MTORC1 and reenter the cell cycle. DDIT4 regulates paligenesis stage 1 by suppressing MTORC1, which leads to autophagy induction. As DDIT4 decreases during paligenesis, TRP53 increases and continues to suppress MTORC1. Cells are licensed to reenter the cell cycle only once IFRD1 suppresses TRP53.

secreting cells that undergo paligenesis after certain injuries. DDIT4 increases during stage 1, concomitant to the MTORC1 nadir, and decreases later as MTORC1 reactivates. Loss of *Ddit4* leads to persistent MTORC1 activity during stage 1, which leads in turn to a dramatic decrease in lysosome and autophagosome induction. Subsequently, *ddit4*<sup>-/-</sup> cells reenter the cell cycle in stage 3 earlier and more frequently than wild-type cells, because the cell cycle reentry is governed by increasing MTORC1, and *ddit4*<sup>-/-</sup> cells constitutively maintain MTORC1 activity.

The second key player in MTORC1 suppression is TRP53. As DDIT4 decreases during paligenesis, TRP53 suppresses MTORC1. Cells reenter the cell cycle only once they also suppress TRP53. IFRD1, the other paligenesis-dedicated protein, is required for TRP53 suppression and cell cycle entry. Thus, DDIT4 and IFRD1 work together to license only healthy cells to reenter the cell cycle. *ddit4*<sup>-/-</sup> cells are inappropriately licensed and carry DNA damage into the S-phase of mitosis, because the block on cell cycle reentry mediated by TRP53 and alleviated by IFRD1 occurs only upon MTORC1 reactivation, not if MTORC1 is already active. Thus, paligenosis affords tissues an abundant and lifelong supply of potential stem cells to tap for repair. But it also comes with molecular safety mechanisms to combat the increased risk for tumorigenesis inherent to allowing long-lived cells to undergo multiple rounds of division and redifferentiation that can cause accumulation and unmasking of somatic mutations.

Thus, we speculate that tissue regeneration and tumorigenesis may both be mediated by the same conserved cellular

program in which autophagy plays a critical initiating role. That tumors may form from aberrant paligenotic regeneration allows for additional predictions about how malignant cells even in already established tumors behave. If tumors arise via aberrant paligenosis, they might perpetuate an abnormal paligenotic response to stress. For example, chemoradiation therapy (CRT) might be interpreted by tumor cells as a paligenosis-inducing injury. In response to CRT, cancer cells may have a low threshold to undergo paligenosis to survive, because the paligenotic process would be largely abnormal in cancer cells already bearing DNA mutation(s). Thus, targeting paligenosis – for example by targeting the specialized massive autophagic steps in the process – might be a promising strategy for cancer treatment. Targeting paligenosis might lead to fewer side effects than targeting mitosis the way CRT does, as paligenosis does not occur during normal organ homeostasis, and is dispensable for the constitutively active, normal organ stem cells damaged by CRT.

## Disclosure statement

The authors declare that they have no conflicts of interest.

## Funding

ZFM was funded by National Natural Science Foundation of China [82072724], Department of Education of Liaoning Province [QNZR2020004]; ZNW by National Key R&D Program of China [MOST-2017YFC0908300], National Natural Science Foundation of China [81961128026, U1908207], Major Scientific and Technological Special Project of Liaoning Province of China [2019020176-JH1/103]; C.J.C. by [T32-CA009547(NIH NCI)]; JCM by [DK094989, DK105129, CA246208, CA239645, and DK110406], and NIH National Cancer Institute [P30 CA091842].

## ORCID

Zhen-Ning Wang  <http://orcid.org/0000-0003-0557-3097>  
Jason C. Mills  <http://orcid.org/0000-0002-0402-4662>

## Reference

- [1] Miao Z-F, Lewis MA, Cho CJ, et al. A dedicated evolutionarily conserved molecular network licenses differentiated cells to return to the cell cycle. *Dev Cell.* 2020;55(2):178–194.e7.