Cell
Leading Edge

Letter

The information theory of aging has not been tested

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A recent article in *Cell* claimed that

> using inducible changes to the epigenome, we find that the act of faithful DNA repair advances aging at physiological, cognitive, and molecular levels, including erosion of the epigenetic landscape, cellular exdifferentiation, senescence, and advancement of the DNA methylation clock, which can be reversed by OSK-mediated rejuvenation. These data are consistent with the information theory of aging, which states that a loss of epigenetic information is a reversible cause of aging.[1]

In this work, Dr. Sinclair and colleagues induced whole-mouse expression of homing endonuclease I-*Ppo*I to prematurely age mice. The corresponding author published two papers—neither cited—showing that I-*Ppo*I targeted to specific cell types is mutagenic, cytotoxic, and progeric, thereby accounting for the progeric effects that they attributed to faithful DNA repair. The corresponding author declined to provide requested data[2] on mice in the 30 days after I-*Ppo*I treatment was concluded or to provide data in support of the statement that "fortunately, it is now apparent that mammals retain a back-up copy of youthful epigenetic information that can safely restore the function of old tissues, akin to reinstalling software."

In their paper published in *Nucleic Acids Research*, Dr. Sinclair and coworkers targeted I-*Ppo*I to T cells for 3 days.[3] This resulted in a ~1-log killing of CD4 and CD8 double-positive cells in the spleen and a similar degree of killing of double-positive T cells in the thymus that was comparable to killing caused by 3 Gy of radiation and was clearly mutagenic to surviving cells (Figure 2).[3] Transcriptional responses to I-*Ppo*I "revealed 'p53 signaling' (mmu04115) as the top overrepresented KEGG pathway ($p = 8.5 \times 10^{-5}$), and 'response to DNA damage stimulus' (GO:0006974) as well as 'induction of apoptosis by intracellular signals' (GO:0008629) as significantly enriched gene ontology terms."[3] This work clearly established that I-*Ppo*I expressed *in vivo* is highly effective at eliminating cells.

In another paper, published in *Developmental Cell*,[4] the co-first-author of the *Cell* paper and Dr. Sinclair were part of a team that targeted I-*Ppo*I to epidermal stem cells using a Krt14-CreER driver and called the resulting mice "iDSB" mice, for "induced double strand breaks."[4] They reported that

> while the ratio of GFP-positive basal cells in reporter control (Krt14-CreER/+; Rosa-H2BGFP/+) mice remained stable for a month, almost all GFP-positive cells (95.14% ± 1.71%) were lost in iDSB mice. Further, histological analysis also showed that I-*Ppo*I-expressing cells that sparsely populated the basal layer at day 3 had strikingly disappeared by day 31 (Figure 2C).

The paper goes on to show that cells with iDSBs mount a p53 response, lose the ability to function as stem cells, and are eliminated,[4] concluding that their "pioneering studies revealed that genotoxic stress and chronological aging abrogate self-renewal of stem cells in hair follicles, skin appendage organs, by triggering their aberrant differentiation, causing hair graying and hair thinning."[4] Indeed, what they described—a cell elimination mechanism of aging—is supported by multiple publications going back through 16 years of aging research.[5] If Dr. Sinclair and coworkers used I-*Ppo*I to kill one cell population and reported a premature aging phenotype, why was cell elimination not examined by the same authors as a progeric mechanism in *Cell*? The cytotoxic and progeric mechanism of I-*Ppo*I[1] was not investigated by Dr. Sinclair's team in their *Cell* paper, and, unfortunately, the relevant *Developmental Cell* or *Nucleic Acids Research* papers mentioned above were not cited either even though they should have been. In our opinion, the missing data and citations may have made it difficult for *Cell* reviewers to evaluate the claims of aging being caused by faithful DNA repair versus an undisclosed and previously established progeric mechanism.

In prior papers, I-*Ppo*I activity was only targeted to specific cell types[3,4] and was activated by subcutaneous 3-day dosing of tamoxifen. The authors of the *Cell* paper used the ubiquitin promoter to target I-*Ppo*I throughout the mouse body for 3 weeks[1] and delivered tamoxifen orally.[6] Figure S3A establishes that ~100% of cells in muscle, liver, and two brain regions were exposed to the tamoxifen-induced transgene.[1] The corresponding author's prior work shows that p53 induction and cell death peak in the days after tamoxifen removal and that cell death has been cleared within a month after tamoxifen removal. Unfortunately, Dr. Sinclair and coworkers provided no analysis of any mouse tissue until a month after tamoxifen removal, thereby missing the entire genotoxic window. We asked Dr. Sinclair for p53-induction and cell-death data within the first 30 days after tamoxifen removal but were not provided with any. Instead, he pointed to Supplementary Figures 1, 2, and 3, which were performed at one month after tamoxifen removal.[2] Because cell death occurred long before this time point, molecular profiling of tissue one month after tamoxifen removal represents a survivorship sampling bias and does not represent cells one could describe as having experienced merely "the act of faithful DNA repair."

Nondisclosure of papers showing the genotoxic and cytotoxic mechanism of I-*Ppo*I is a problem. Had these papers been cited, one would expect reviewers to

ask for data examining p53 induction and cell elimination at time points within the first month after tamoxifen removal. Figure S3A shows that essentially all nuclei in the liver, muscle, hippocampus, and cortex show stop cassette removal, which means exposure to I-*Ppo*I was not mild over 21 days. Though Dr. Sinclair replied to us that levels of tamoxifen were intended to be "very low,"[2] the literature indicates that oral dosing of tamoxifen results in substantially higher concentration of the active metabolite 4-hydroxytamoxifen than subcutaneous dosing.[6] There does not appear to be a paper in the literature in which I-*Ppo*I was expressed for a longer period or at a higher dose than what was done in *Cell*. A more-than-two-decade literature on I-*Ppo*I, including two papers from Dr. Sinclair, established genotoxic cell elimination as the result of I-*Ppo*I expression.[3,4,7] It was surprising that the authors can provide no data on genotoxicity when they earlier wrote

> consistent with cell-intrinsic epigenetic deregulation being a minor consequence of continued DSB exposure *in vivo*, a recent study shows that DNA damage-induced, age-associated functional decline can be attributed in large part to systemic consequences of DSBs, including cell death, tissue atrophy, and the ensuing, non-cell-autonomous inflammatory response.[3]

The *Cell* paper continued, "to further rule out mutations … and gain further insight into epigenetic alterations as a cause of aging, we tested the effect of resetting the epigenome *in vitro* and *in vivo*." However, if one has a hypothesis, one is expected to test it versus reasonable alternatives—especially the null hypothesis—and not against an obviously false straw man. The null hypothesis to be tested here is that a 3-week whole-body iDSB treatment killed lots of cells, causing tissue and systemic inflammatory changes responsible for accelerated aging. Looking for mutations long after cells have been eliminated does not meaningfully test the authors' hypothesis. Figure 2C in the concurrently submitted *Developmental Cell* paper shows that iDSBs lead to cell elimination within one month and cause a prematurely aged phenotype,[4] but the *Cell* paper does not report any analysis for one month. At one month after withdrawal of tamoxifen, the *Cell* paper shows that I-*Ppo*I sites are no longer cut and that they cannot find mutations.[1] However, based on what is known about genotoxic cell death, one would predict that multiple—potentially all—tissues were damaged by loss of cells creating complex, inflammatory, and progeric health problems, such as were reported in *Developmental Cell* with I-*Ppo*I targeted to a single cell type.[3] This mechanism accounts for the phenotypes shown in Figures 2, 3, and 4.[1] We further documented missing controls and outlined a fair test of the information theory in one model of aging.[2]

The effect of Yamanaka factors on the epigenetic state of cells is well known,[8] but the *Cell* paper's highlights told readers that "aging can be driven forward and backward." Their discussion concluded that "fortunately, it is now apparent that mammals retain a back-up copy of youthful epigenetic information that can safely restore the function of old tissues, akin to reinstalling software." Moreover, their graphical abstract showed an old mouse on the right driven by OSK to a young mouse on the left. However, the paper showed no data on functional rejuvenation due to OSK treatment. Figure 7S shows the effect on the eyes of the tamoxifen-induced I-*Ppo*I: the eyes are opaque in the I-*Ppo*I mice but there was no functional characterization of OSK-treated iDSB mice. The *Cell* paper does not document any old tissue with restored function in any mouse and no data in support of restoring function were provided by Dr. Sinclair when requested.[2] Given their related manuscripts not being cited and appropriately cross-referenced, the issues around I-*Ppo*I-induced cell death, and lack of demonstration of the major claims of the paper, we remain concerned about the validity of the published *Cell*[1] paper.

DECLARATION OF INTERESTS

J.A.T. is the owner of Augur Precision Medicine LTD. C.B. has ownership and advisory interests in ChromaDex, Alphina Therapeutics, and Juvenis.

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