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REJUVENATING HALLMARKS OF AGEING BY MODULATING PROTEOSTASIS AND MITOCHONDRIAL ACTIVITY

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Abstract

Introduction. Aging is characterized by cellular decline driven by hallmark processes such as loss of proteostasis and mitochondrial dysfunction. These impairments contribute to toxic protein accumulation, oxidative stress, and tissue degeneration. While transient expression of reprogramming factors (Oct4, Sox2, Klf4, and c-Myc; OSKM) has been proposed to reverse age-associated dysfunction, the precise temporal dynamics of rejuvenation remain unclear.

Aim. The study aims to determine the temporal windows of proteostasis and mitochondrial function restoration in senescent human fibroblasts during partial OSKM reprogramming.

Materials and Methods. Senescent human lung fibroblasts were engineered to express OSKM under doxycycline control. Restoration of proteostasis was assessed using the Proteasome-Glo™ Cell-Based assay to measure chymotrypsin-like, trypsin-like, and caspase-like proteasomal activities. Mitochondrial activity was evaluated through measurement of reactive oxygen species using MitoSOX Red dye and flow cytometry. Data were analyzed by comparing induced and senescent control cells, with statistical testing to establish significance.

Results. OSKM induction led to a time-dependent restoration of proteostasis. By day 5, proteasomal activities were significantly elevated in OSKM-induced cells compared to senescent controls (chymotrypsin-like activity increased from 48,976 to 128,078 RLU; trypsin-like activity from 12,222 to 42,333 RLU; caspase-like activity from 32,614 to 126,628 RLU). Mitochondrial rejuvenation occurred later, with ROS levels significantly reduced by day 10, reaching values comparable to young fibroblasts ($p < 0.001$). These results highlight distinct temporal windows for the recovery of proteostasis and mitochondrial function.

Conclusion. Partial reprogramming via transient OSKM expression can effectively reverse key hallmarks of aging in senescent fibroblasts. Proteostasis is restored within 5 days, followed by mitochondrial functional recovery at 10 days, suggesting a sequential rejuvenation process. These findings provide critical temporal insights for optimizing reprogramming-based interventions and support the development of clinically translatable rejuvenation strategies.

Keywords: proteostasis, mitochondrial dysfunction, aging, reprogramming factors.

Introduction. Aging is a complex, multifactorial biological process characterized by the gradual decline in cellular and tissue homeostasis, increased vulnerability to stressors, and a higher incidence of chronic diseases such as neurodegeneration, cardiovascular dysfunction, metabolic syndromes, and cancer [1, 2]. At the cellular level, aging manifests through a series of well-established hallmarks, including genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, and stem cell exhaustion [3]. Among these, loss of proteostasis and

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mitochondrial dysfunction are two interlinked features that critically impair cellular function and contribute to the decline in tissue resilience observed with age.

Proteostasis, short for protein homeostasis, is the intricate cellular network responsible for ensuring proper protein folding, trafficking, and degradation [4]. Aging disrupts this network through a combination of factors: reduced activity of proteasomes and autophagy pathways, accumulation of misfolded or damaged proteins, and chronic activation of stress response pathways. These disturbances can lead to the buildup of toxic protein aggregates, such as amyloid fibrils and lipofuscin, which are common features of aged and diseased tissues.

Mitochondria, the cellular powerhouses, are equally susceptible to age-related dysfunction. Mitochondrial DNA damage, impaired oxidative phosphorylation, increased production of reactive oxygen species (ROS), and defective mitophagy all contribute to cellular energy deficits and redox imbalance [5]. Importantly, mitochondria and proteostasis are functionally intertwined; misfolded proteins can impair mitochondrial function, while mitochondrial ROS exacerbate protein damage, creating a vicious cycle of cellular decline.

In recent years, the field of regenerative medicine and gero-science has turned to cellular reprogramming as a potential intervention for reversing aging phenotypes. The transient induction of Yamanaka factors (*Oct4*, *Sox2*, *Klf4*, and *c-Myc* (OSKM)) has shown promise in restoring youthful cellular characteristics without inducing full pluripotency [6]. This process, known as partial reprogramming, has been demonstrated to reset epigenetic markers, restore mitochondrial integrity, reduce senescence-associated features, and improve overall cellular function in both in vitro and in vivo models. However, despite these promising outcomes, the precise dynamics and timing of functional recovery remain poorly defined, and concerns persist regarding the risks of genomic instability and tumorigenicity associated with genetic reprogramming.

To address these limitations, recent studies have explored chemical reprogramming strategies using small molecules that modulate epigenetic regulators, metabolic pathways, and signaling cascades involved in aging and cellular plasticity [7]. One such compound is valproic acid (VPA), a well-known histone deacetylase (HDAC) inhibitor, which has demonstrated the ability to enhance reprogramming efficiency, promote neuronal differentiation, and improve stem cell engraftment [8]. VPA's impact on gene expression through chromatin remodeling positions it as a potential non-genetic tool for modulating aging hallmarks, including proteostasis and mitochondrial health.

In this study, we set out to dissect the temporal progression of rejuvenation in two interconnected aging hallmarks, proteostasis and mitochondrial function, following partial reprogramming via OSKM induction in senescent human lung fibroblasts. By mapping out the timeline of molecular recovery and identifying optimal windows for intervention, our work contributes to a deeper understanding of how to harness reprogramming for therapeutic purposes. These insights may serve as a foundation for the development of precise, time-sensitive, and clinically translatable rejuvenation strategies that go beyond disease management and aim to restore cellular vitality at its root.

Materials and Methods.

Timing of rejuvenation of proteostasis activity

We measured restoration of proteostasis in senescent human lung fibroblast cells after induction of reprogramming factors using a commercial assay kit of proteasome activity Proteasome-Glo™ Cell-Based from Promega (G1180). The kit measures chymotrypsin-like, trypsin-like and caspase activity a protease associated with the proteasome complex in cultured cells. The kit uses peptide substrates Suc-LLVY-aminoluciferin, Z-LRR-aminoluciferin and Z-LPnLD-aminoluciferin to measure proteasomal activity. The increase of luminescence intensity indicates increased proteostasis activity. Two groups of cells were used for the experiment

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fibroblasts. First group, control cells of senescent fibroblasts. The second group, cells in which reprogramming factors were induced for 5 days.

Timing of rejuvenation of mitochondrial activity

We have assayed mitochondrial function by measuring reactive oxygen species. To measure reactive oxygen species (ROS), MitoSOX Red dye (cat number: M36008) was used. MitoSOX Red accumulates predominantly in mitochondria where it is oxidized by superoxide, a type of reactive oxygen species, and exhibits red fluorescence. Flow cytometry Attune NxT was used to measure the fluorescence of MitoSOX Red in cells. MitoSOX Red was excited with a laser of 488 nm wavelength, the degree of fluorescence could be measured with an emission filter tuned to the 590/40 nm wavelength. After measuring the fluorescence of samples, the raw data (FSC files) was extracted and the FlowJo program was used to analyse the raw data. The median fluorescence intensity of cells from different stages of reprogramming was compared and using a statistical test (two tailed t-test), the degree of significance of the ROS difference in the cells was reported (Figure 1, 2).

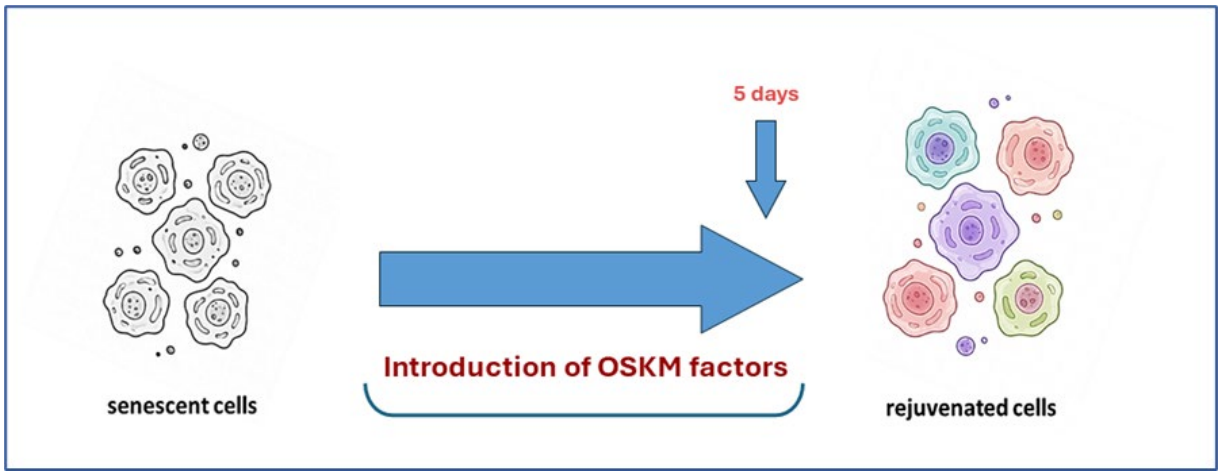

Figure 1. Method for rejuvenation of proteostasis activity

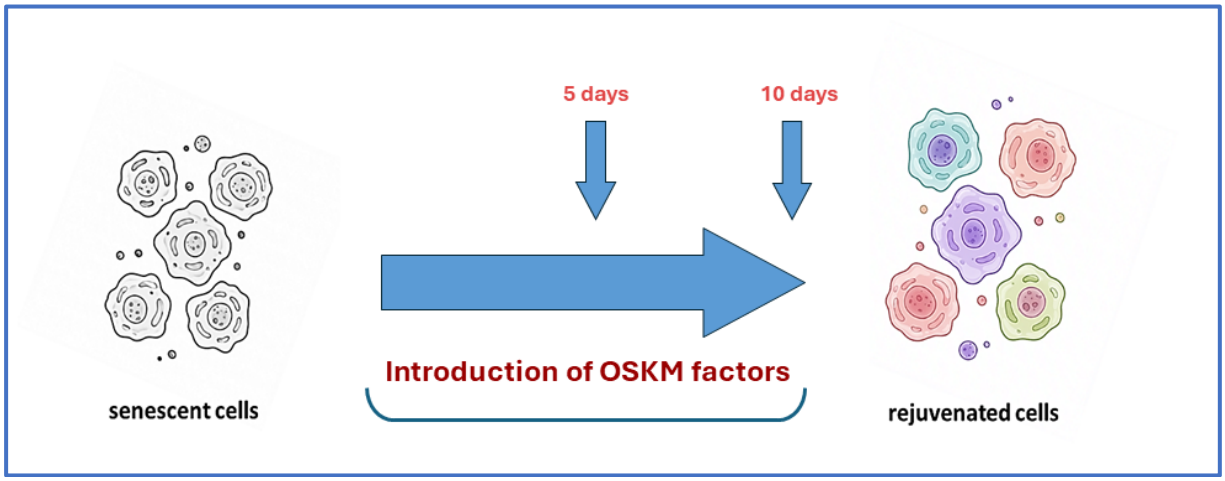

Figure 2. Method for rejuvenation of mitochondrial activity

Senescent Fibroblast Cultivation and OSKM expression

Primary human lung fibroblasts [9] were engineered to express OSKM after exposure to *doxycycline* (*dox*) [10] were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific), 1% penicillin-

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streptomycin (Gibco), and 1% GlutaMAX (Gibco), maintained at 37°C in a humidified atmosphere with 5% CO_2. To induce replicative senescence, fibroblasts were serially passaged until they reached passage 25–26, at which point they exhibited classical senescence-associated morphological changes (enlarged and flattened shape), reduced proliferation rate, and increased senescence-associated β-galactosidase (SA-β-gal) staining. Cells from passage 15 were used as «young» controls.

Senescent phenotype was confirmed prior to reprogramming experiments by assessing cell cycle arrest, SA-β-gal staining (using the Senescence β-Galactosidase Staining Kit, Cell Signaling Technology), and increased expression of $p16^{INK4a}$ and $p21^{CIP1/WAF1}$ via Western blotting. Cells were seeded at a density of 2×10^5 cells/well in 6-well plates for all downstream assays, including OSKM induction, proteasome activity measurement, and ROS detection.

Results.

Rejuvenation of Proteostasis Function

We first evaluated the temporal dynamics of proteasomal activity recovery in senescent fibroblasts following the induction of OSKM reprogramming factors. Using the Proteasome-Glo™ Cell-Based assay, we measured chymotrypsin-like, trypsin-like, and caspase-like proteasomal activities at 5 days post-induction. Senescent fibroblasts without OSKM expression exhibited markedly reduced proteasomal activity compared to negative control (young) cells. However, cells treated with OSKM showed a substantial and time-dependent increase in proteasomal function.

By day 5, all three enzymatic activities showed significant elevation compared to senescent controls: chymotrypsin-like activity increased from 48,976 RLU to 128,078 RLU; trypsin-like activity rose from 12,222 RLU to 42,333 RLU; and caspase-like activity increased from 32,614 RLU to 126,628 RLU (Table 1 and Figure 1). These findings indicate that OSKM-mediated partial reprogramming results in a robust and progressive restoration of proteostasis within senescent cells.

Table 1. Proteasomal activity in old cells and rejuvenated cells after OSKM expression (graphical representation in Figure 1).

Samples	Chymotrypsin-like activity	Trypsin-like activity	Caspase-like activity
Senescent cells	48,976	12,222	32,614
After 5 days	128,078	42,333	126,628

*Measurement units – Relative Luminescence Units or RLU.

Proteasomal activity, including chymotrypsin-like, trypsin-like, and caspase-like functions, was evaluated in senescent and young fibroblasts. Proteolytic activity in senescent fibroblasts was significantly reduced compared to young cells, as demonstrated by the decrease in chymotrypsin-like protease activity. After the induction of reprogramming factors for 5 days, senescent fibroblasts exhibited a marked increase in proteasomal activity relative to untreated controls. The experiment was independently repeated twice, and all samples were measured in triplicate (Figure 3).

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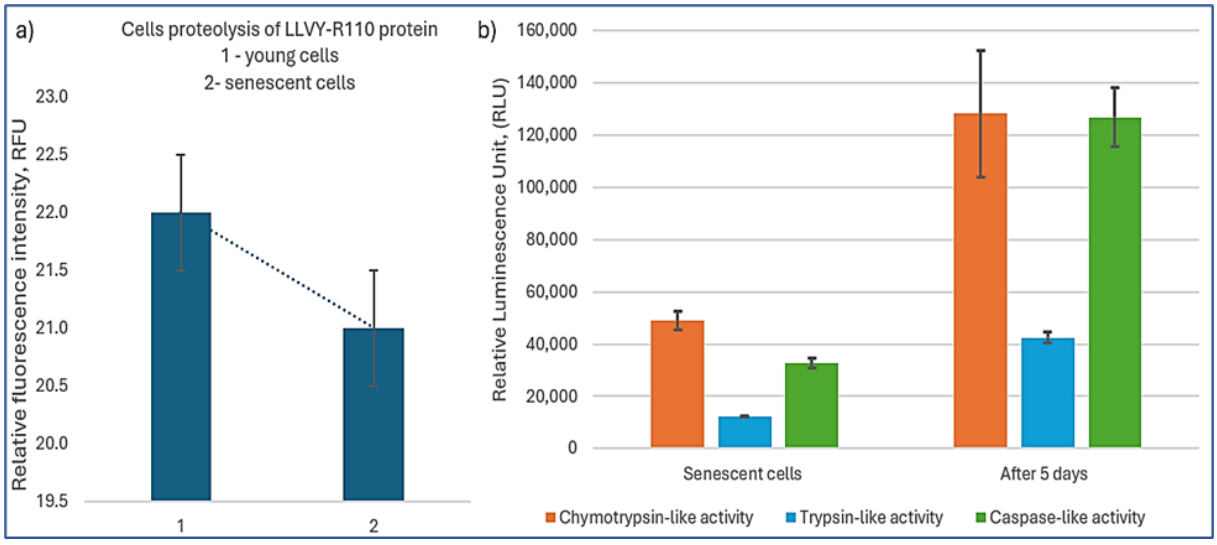

Figure 3. Rejuvenation of proteasomal activity production in senescent primary lung fibroblasts after dox-induction

Rejuvenation of Mitochondrial Function

To assess mitochondrial function during the reprogramming time course, we quantified reactive oxygen species (ROS) levels using MitoSOX Red dye and flow cytometry. In senescent primary lung fibroblasts (passage 26), ROS production was significantly higher than in young fibroblasts (passage 15), confirming age-associated mitochondrial dysfunction. Upon induction of OSKM, ROS levels significantly decreased by day 10, suggesting mitochondrial rejuvenation. In particular, the ROS levels decreased by 23.9 % compared to baseline senescent cells, reaching levels statistically indistinguishable from young controls.

Specifically, median fluorescence intensity (MFI) values for MitoSOX Red declined in OSKM-induced cells by day 10, reaching levels statistically indistinguishable from those in young controls ($p < 0.001$, paired t-test; Figure 4). This temporal reduction in ROS indicates that mitochondrial function begins to normalize approximately 10 days post-OSKM induction**,** providing a defined window for further therapeutic interventions aimed at enhancing mitochondrial recovery.

The y-axis for both graphs indicates the averaged value of median fluorescence intensity (MFI) after background subtraction. (a) ROS production was significantly higher in old (P26) primary lung fibroblasts compared to young (P15) cells (n=3). (b) Upon the induction of OSKM factors the ROS production significantly decreased on the 10^{th} day (n=3). Data are presented as mean ± SD; paired t-test. ***$p < 0.001$.

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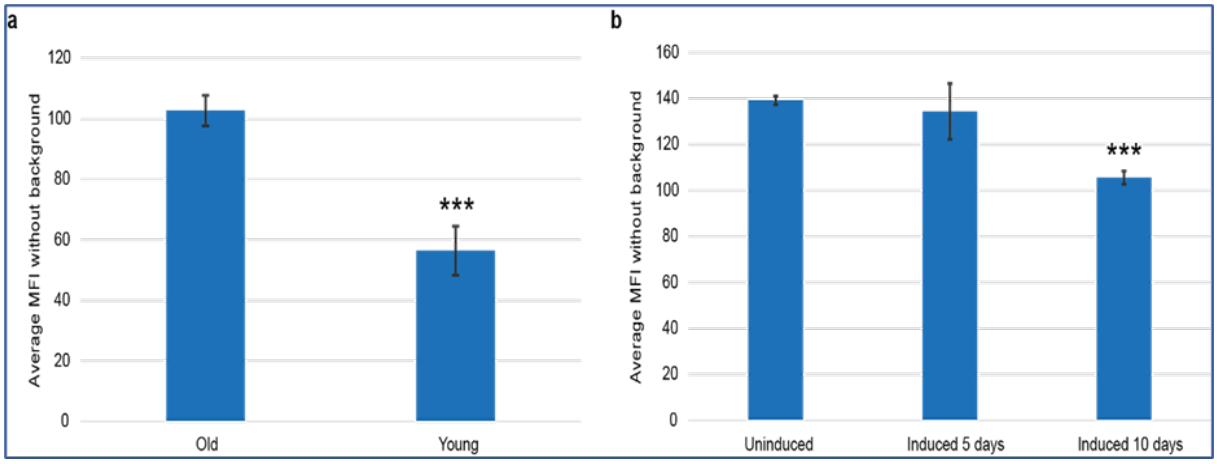

Figure 4. Amelioration of reactive oxygen species (ROS) production in senescent primary lung fibroblasts after *dox*-induction.

Under our conditions, mitochondrial restoration occurs 10 days after OSKM expression, establishing a critical temporal marker for reprogramming-induced rejuvenation. To enhance this process, the selected compounds (listed below) will be administered on day 10 following reprogramming initiation. This intervention aims to accelerate mitochondrial recovery, restore cellular function, and ultimately promote longevity.

Discussion. Cellular reprogramming has emerged as a powerful tool for redefining cell identity, enabling the generation of specific cell types for therapeutic applications [9]. Traditionally, this process has been driven by intrinsic mechanisms, such as exposure to oocyte cytoplasm or the introduction of transcription factors, which can revert somatic cells to a pluripotent state [10]. While effective, these approaches often involve genetic modifications, raising safety, efficiency, and scalability concerns. Alternatively, chemical reprogramming using small molecules offers a precise, highly controllable, and non-genetic strategy to manipulate cell fate, presenting a more practical avenue for regenerative medicine [11].

This study demonstrates that partial cellular reprogramming through OSKM induction can initiate measurable rejuvenation of two key aging hallmarks, proteostasis and mitochondrial function, in senescent human lung fibroblasts. Importantly, we show that these improvements follow a distinct temporal sequence: proteasomal activity begins to recover by day 5 post-induction, while mitochondrial function, indicated by reduced ROS levels, improves by day 10. These results reinforce the emerging concept that partial reprogramming does not reset cellular identity indiscriminately but rather activates targeted, time-dependent repair mechanisms that can be modulated for therapeutic benefit.

The recovery of proteostasis, as evidenced by enhanced proteasomal activity, suggests reactivation of cellular systems responsible for degrading damaged or misfolded proteins. This is critical, as the age-related decline in proteasome efficiency is known to promote the accumulation of toxic aggregates that impair cellular function and increase vulnerability to neurodegenerative diseases [12]. Our data indicate that partial reprogramming may restore the balance between protein synthesis and degradation without driving cells into a dedifferentiated or tumorigenic state. This aligns with previous studies showing that transient OSKM expression can rejuvenate epigenetic markers and improve stress resistance in aged cells [13-15].

Mitochondrial rejuvenation, observed approximately 10 days post-OSKM induction, further supports the role of reprogramming in reversing age-associated oxidative stress and metabolic decline. A reduction in mitochondrial ROS implies not only improved mitochondrial quality but also potential restoration of mitophagy and biogenesis pathways. Given the critical

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interplay between mitochondria and proteostasis, hereby dysfunctional mitochondria contribute to protein oxidation, and impaired proteostasis disrupts mitochondrial homeostasis, our findings highlight a mutually reinforcing loop of rejuvenation that could serve as a core therapeutic target in age-related disease.

An important consideration is the translational applicability of our findings. While the present study demonstrates clear rejuvenation of proteostasis and mitochondrial function in vitro, the extent to which these effects can be reproduced in vivo remains uncertain. In animal models, transient OSKM expression has been shown to enhance tissue regeneration and extend lifespan; however, concerns about incomplete reprogramming, tumorigenic risk, and heterogeneity of cellular responses remain barriers to clinical translation. Moreover, the controlled induction of reprogramming factors in cultured fibroblasts does not fully recapitulate the complexity of aged tissues, where multiple cell types interact within a pro-inflammatory and metabolically stressed microenvironment. For clinical use, strategies that allow precise temporal control of OSKM or chemical alternatives that mimic their effects will be necessary to ensure both efficacy and safety. Thus, while our data provide proof-of-principle that hallmarks of aging can be rejuvenated, considerable work is needed to adapt these interventions into clinically viable therapies.

The temporal aspect of our findings is particularly relevant for developing clinically viable rejuvenation strategies. While full reprogramming carries a risk of loss of cell identity or teratoma formation, transient, time-bound interventions may allow us to selectively rejuvenate cells without pushing them beyond the point of safety. Our results suggest that even brief reprogramming pulses can produce sustained physiological benefits, provided they are applied within an optimized time

Despite these promising outcomes, several limitations should be acknowledged. First, our model is limited to fibroblasts, and it remains unclear whether the observed timeline of rejuvenation would generalize across other cell types, particularly those with slower turnover such as neurons or cardiomyocytes. Second, while we tracked two important hallmarks of aging, a more comprehensive evaluation, encompassing telomere length, DNA damage, senescence-associated β-galactosidase activity, and epigenetic age, would provide a fuller picture of rejuvenation dynamics. Finally, the long-term effects of repeated or cyclic partial reprogramming and chemical treatment on genomic stability and cancer risk warrant further investigation.

In summary, our study provides mechanistic and temporal insights into how partial reprogramming and epigenetic modulation can reverse specific aging hallmarks. The ability to restore proteostasis and mitochondrial function in a coordinated, time-sensitive manner suggests a path forward for targeted interventions that promote cellular resilience and delay functional decline. These findings support the emerging paradigm of rejuvenation medicine, where aging is no longer viewed as an unmodifiable fate, but rather as a dynamic process amenable to intervention and control.

Limitations of the Study. Despite the promising findings, several limitations should be considered when interpreting our results. First, the induction of OSKM factors, even when transient, may trigger unintended side effects. Previous studies have reported that partial reprogramming can alter cellular proliferation rates, induce aberrant gene expression, and in some cases, predispose cells to epigenetic instability or loss of lineage identity. Although we observed no overt signs of dedifferentiation under our experimental conditions, the possibility of subtle genomic or epigenomic perturbations cannot be excluded. These risks underscore the importance of optimizing induction protocols to strike a balance between rejuvenation benefits and safety concerns.

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Second, the scope of measured endpoints in this study was restricted to proteasomal activity and mitochondrial function as assessed by ROS production. While these markers are representative of two key hallmarks of aging, they do not fully capture the complexity of the rejuvenation process. Critical parameters such as telomere length, DNA damage, chromatin accessibility, and global transcriptomic changes were not evaluated. Likewise, functional endpoints, including long-term proliferative capacity or differentiation potential, remain unexplored. Future studies will need to integrate these additional markers to provide a more comprehensive understanding of rejuvenation dynamics and associated risks.

Finally, our experimental design was limited to human lung fibroblasts. The extent to which the observed timeline of rejuvenation applies to other primary human cell types, especially post-mitotic cells such as neurons or cardiomyocytes, remains uncertain.

Conclusion. Loss of proteostasis activity is a primary hallmark of aging [3]. Past research has shown that OSKM-driven age reprogramming rejuvenates proteostasis loss in old/senescent wild-type mouse and human fibroblasts [16, 17], but the exact time of rejuvenation has not been measured. The loss of mitochondrial function is a secondary hallmark of aging [18, 19]. This study provides compelling evidence that transient expression of reprogramming factors (OSKM) can effectively reverse age-associated deterioration in proteostasis and mitochondrial function. Notably, proteasomal activity is significantly restored within 5 days, and mitochondrial rejuvenation occurs by day 10. These findings underscore the importance of defining temporal parameters in reprogramming-based interventions. In addition, integration of chemical approaches [15] with partial reprogramming protocols may offer a scalable, safer, and more clinically viable path toward therapeutic rejuvenation.

Conflicts of interest.
The authors declare no conflict of interest.
Authors' contribution.
N. Berdigaliyev– experimental work, review and editing.
P.B. Singh –writing, review and editing, supervision, project administration.
We declare that this material has not been previously submitted for publication in other publications and is not under consideration by other publishers.
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REFERENCES

1. Tenchov R., Sasso J. M., Wang X., Zhou Q. A. Aging hallmarks and progression and age-related diseases: a landscape view of research advancement // ACS Chemical Neuroscience. – 2023. – Т. 15, № 1. – С. 1–30. – DOI: 10.1021/acschemneuro.3c00531.
2. Guo J., Huang X., Dou L., Yan M., Shen T., Tang W., Li J. Aging and aging-related diseases: from molecular mechanisms to interventions and treatments // Signal Transduction and Targeted Therapy. – 2022. – Т. 7, № 1. – DOI: 10.1038/s41392-022-01251-0.
3. López-Otín C., Blasco M. A., Partridge L., Serrano M., Kroemer G. Hallmarks of aging: an expanding universe // Cell. – 2023. – Т. 186, № 2. – С. 243–278. – DOI: 10.1016/j.cell.2022.11.001.
4. Shukla M., Narayan M. Proteostasis and its role in disease development // Cell Biochemistry and Biophysics. – 2024. – Т. 83, № 2. – С. 1725–1741. – DOI: 10.1007/s12013-024-01581-6.

ISSN 2524 – 0684 e-ISSN 2524 – 0692

5. Zong Y., Li H., Liao P., Chen L., Pan Y., Zheng Y., Zhang C., Liu D., Zheng M., Gao J. Mitochondrial dysfunction: mechanisms and advances in therapy // Signal Transduction and Targeted Therapy. – 2024. – Т. 9, № 1. – DOI: 10.1038/s41392-024-01839-8.
6. Aguirre M., Escobar M., Forero Amézquita S., Cubillos D., Rincón C., Vanegas P., Tarazona M. P., Atuesta Escobar S., Blanco J. C., Celis L. G. Application of the Yamanaka transcription factors Oct4, Sox2, Klf4, and c-Myc from the laboratory to the clinic // Genes. – 2023. – Т. 14, № 9. – DOI: 10.3390/genes14091697.
7. Pereira B., Correia F. P., Alves I. A., Costa M., Gameiro M., Martins A. P., Saraiva J. A. Epigenetic reprogramming as a key to reverse ageing and increase longevity // Ageing Research Reviews. – 2024. – Т. 95. – DOI: 10.1016/j.arr.2024.102204.
8. Cha H., Lee J., Park H. H., Park J. H. Direct conversion of human fibroblasts into osteoblasts triggered by histone deacetylase inhibitor valproic acid // Applied Sciences. – 2020. – Т. 10, № 20. – DOI: 10.3390/app10207372.
9. Pitrez P. R., Monteiro L. M., Borgogno O., Nissan X., Mertens J., Ferreira L. Cellular reprogramming as a tool to model human aging in a dish // Nature Communications. – 2024. – Т. 15, № 1. – DOI: 10.1038/s41467-024-46004-5.
10. Swegen A., Appeltant R., Williams S. A. Cloning in action: can embryo splitting, induced pluripotency and somatic cell nuclear transfer contribute to endangered species conservation? // Biological Reviews. – 2023. – Т. 98, № 4. – С. 1225–1249. – DOI: 10.1111/brv.12951.
11. Zhu F., Nie G. J. Cell reprogramming: methods, mechanisms and applications // Cell Regeneration. – 2025. – Т. 14, № 1. – DOI: 10.1186/s13619-025-00229-x.
12. Weinberg J., Gaur M., Swaroop A., Taylor A. Proteostasis in aging-associated ocular disease // Molecular Aspects of Medicine. – 2022. – Т. 88. – DOI: 10.1016/j.mam.2022.101157.
13. Rosenzweig-Lipson S. Opportunities for cellular rejuvenation in Alzheimer's disease: how epigenetic reprogramming and chaperone-mediated autophagy are enabling next generation therapeutic approaches // Journal of Prevention of Alzheimer's Disease. – 2023. – Т. 10, № 4. – С. 661–668. – DOI: 10.14283/jpad.2023.106.
14. Berger F. Meiosis as a mechanism for epigenetic reprogramming and cellular rejuvenation // Development. – 2024. – Т. 151, № 20. – DOI: 10.1242/dev.203046.
15. Simpson D. J., Olova N. N., Chandra T. Cellular reprogramming and epigenetic rejuvenation // Clinical Epigenetics. – 2021. – Т. 13, № 1. – DOI: 10.1186/s13148-021-01158-7.
16. Alle Q., Le Borgne E., Bensadoun P., Lemey C., Béchir N., Gabanou M., Estermann F., Bertrand-Gaday C., Pessemesse L., Toupet K., Desprat R., Vialaret J., Hirtz C., Noël D., Jorgensen C., Casas F., Milhavet O., Lemaitre J. M. A single short reprogramming early in life initiates and propagates an epigenetically related mechanism improving fitness and promoting an increased healthy lifespan // Aging Cell. – 2022. – Т. 21, № 11. – DOI: 10.1111/acel.13714.
17. Sarkar T. J., Quarta M., Mukherjee S., Colville A., Paine P., Doan L., Tran C. M., Chu C. R., Horvath S., Qi L. S., Bhutani N., Rando T. A., Sebastiano V. Transient non-integrative expression of nuclear reprogramming factors promotes multifaceted amelioration of aging in human cells // Nature Communications. – 2020. – Т. 11, № 1. – DOI: 10.1038/s41467-020-15174-3.
18. Tartiere A. G., Freije J. M. P., López-Otín C. The hallmarks of aging as a conceptual framework for health and longevity research // Frontiers in Aging. – 2024. – Т. 5. – DOI: 10.3389/fragi.2024.1334261.

ISSN 2524 – 0684 e-ISSN 2524 – 0692

19. López-Otín C., Pietrocola F., Roiz-Valle D., Galluzzi L., Kroemer G. Meta-hallmarks of aging and cancer // Cell Metabolism. – 2023. – Т. 35, № 1. – С. 12–35. – DOI: 10.1016/j.cmet.2022.11.001.

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ҚАРТАЮ БЕЛГІЛЕРІН ПРОТЕОСТАЗ БЕН МИТОХОНДРИЯЛЫҚ БЕЛСЕНДІЛІКТІ МОДУЛЯЦИЯЛАУ АРҚЫЛЫ ЖАСАРТУ

Н. БЕРДИГАЛИЕВ, П.Б. СИНГХ

Биологиялық ғылымдар кафедрасы, Медицина мектебі, Назарбаев Университеті, Астана, Қазақстан

Түйіндеме

Кіріспе. Қартаю протеостаздың бұзылуы және митохондрия дисфункциясы сияқты негізгі үдерістерден туындайтын жасушалық нашарлаумен сипатталады. Бұл бұзылыстар уытты ақуыздардың жиналуына, тотығу стрессіне және тіндердің дегенерациясына ықпал етеді. Репрограммалау факторларының уақытша экспрессиясы (Oct4, Sox2, Klf4 және c-Myc; OSKM) жасқа байланысты дисфункцияны жою үшін ұсынылғанымен, жасару үдерісінің нақты уақыттық динамикасы әлі анық емес.

Мақсаты. Зерттеу OSKM арқылы ішінара репрограммалау кезінде адамның сенесцентті фибробласттарында протеостаз бен митохондриялық функцияның қалпына келуінің уақыттық шектерін анықтауға бағытталған.

Материалдар мен әдістер. Адамның қартаюдағы өкпе фибробласттары доксициклиннің бақылауымен OSKM экспрессиясы үшін модификацияланды. Протеостаздың қалпына келуін Proteasome-Glo™ жасушалық талдауын қолданып, химотрипсинтәрізді, трипсинтәрізді және каспазатәрізді протеасомдық белсенділікті өлшеу арқылы бағалады. Митохондрия белсенділігі MitoSOX Red бояғышын және ағымдық цитометрияны қолдана отырып белсенді оттегі түрлерін өлшеу арқылы анықталды. Деректер OSKM индукцияланған және қартаюдағы бақылау жасушаларын салыстыру арқылы, маңыздылығын анықтау үшін статистикалық өңдеумен талданды.

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Нәтижелер. OSKM индукциясы протеостаздың уақытқа тәуелді қалпына келуіне әкелді. 5-ші күнге қарай OSKM-индукцияланған жасушаларда протеасома белсенділігі қартаюдағы бақылау жасушаларымен салыстырғанда айтарлықтай артты (химотрипсинтәрізді: 48 976-дан 128 078 RLU-ға дейін; трипсинтәрізді: 12 222-ден 42 333 RLU-ға дейін; каспазатәрізді: 32 614-тен 126 628 RLU-ға дейін). Митохондрияның жасаруы кейінірек байқалды, белсенді оттегі түрлерінің деңгейі 10-шы күні айтарлықтай төмендеп, жас фибробласттардағы мәндерге сәйкес болды ($p < 0,001$). Бұл нәтижелер протеостаз бен митохондрия функциясының қалпына келуіндегі әртүрлі уақыт аралықтарын айқындайды.

Қорытынды. OSKM-нің уақытша экспрессиясы арқылы ішінара репрограммалау қартаюдағы фибробласттарда қартаюдың негізгі белгілерін тиімді түрде кері қайтара алады. Протеостаз 5 күн ішінде қалпына келеді, ал 10 күннен кейін митохондрия функционалдығы қайта қалпына келеді, бұл жасару үдерісінің бірізділігін көрсетеді. Бұл нәтижелер репрограммалауға негізделген араласуларды оңтайландыру үшін маңызды уақыттық ақпарат береді және клиникалық қолданыстағы жасарту стратегияларын әзірлеуге ықпал етеді.

Түйінді сөздер: протеостаз, митохондриялық дисфункция, қартаю, қайта бағдарламалау факторлары.

ОМОЛАЖИВАНИЕ ПРИЗНАКОВ СТАРЕНИЯ ПУТЕМ МОДУЛЯЦИИ ПРОТЕОСТАЗА И МИТОХОНДРИАЛЬНОЙ АКТИВНОСТИ

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Аннотация

Введение. Старение характеризуется клеточным ухудшением, вызванным такими ключевыми процессами, как нарушение протеостаза и дисфункция митохондрий. Эти нарушения способствуют накоплению токсичных белков, окислительному стрессу и дегенерации тканей. Хотя временная экспрессия факторов репрограммирования (Oct4, Sox2, Klf4 и c- Myc; OSKM) была предложена для устранения возрастной дисфункции, точная временная динамика омоложения остаётся неясной.

Цель. Исследование направлено на определение временных рамок восстановления протеостаза и митохондриальной функции в сенесцентных фибробластах человека при частичном репрограммировании OSKM.

Материалы и методы. Стареющие фибробласты лёгких человека были модифицированы для экспрессии OSKM под контролем доксициклина. Восстановление протеостаза оценивали с помощью клеточного анализа Proteasome-Glo™ для измерения химотрипсиноподобной, трипсиноподобной и каспазоподобной протеасомной активности. Активность митохондрий оценивали путём измерения активных форм кислородас использованием красителя MitoSOX Red и проточной цитометрии. Данные анализировали путём сравнения индуцированных и стареющих контрольных клеток с использованием статистической обработки для установления значимости.

Результаты. Индукция OSKM привела к зависящему от времени восстановлению протеостаза. К 5-му дню активность протеасом была значительно повышена в клетках, индуцированных OSKM, по сравнению со стареющими контрольными клетками (химотрипсиноподобные: от 48 976 до 128 078 RLU; трипсиноподобные: от 12 222 до 42

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333 RLU; каспазоподобные: от 32 614 до 126 628 RLU). Омоложение митохондрий происходило позже: уровни активных форм кислорода значительно снижались к 10-му дню, достигая значений, сопоставимых с молодыми фибробластами ($p<0,001$). Эти результаты подчеркивают различные временные интервалы для восстановления протеостаза и функции митохондрий.

Заключение. Частичное репрограммирование посредством транзиентной экспрессии OSKM может эффективно обратить вспять ключевые признаки старения в стареющих фибробластах. Протеостаз восстанавливается в течение 5 дней, а затем, через 10 дней, восстанавливается функциональность митохондрий, что свидетельствует о последовательном процессе омоложения. Эти результаты предоставляют важную временную информацию для оптимизации вмешательств, основанных на репрограммировании, и способствуют разработке клинически применимых стратегий омоложения.

Ключевые слова: протеостаз; митохондриальная дисфункция; старение; факторы перепрограммирования.