GMR
The Original

ROLE OF EPIGENETIC ALTERATIONS IN AGE-ASSOCIATED CELLULAR DYSFUNCTION

Dr. Archana[1], Dr. Anandhi D[2], Dr. Shreya Srinivasan[3], Dr. Maharshikumar B. Shukla[4], Preetjot Singh[5]

[1.] Professor, Department of Pathology Meenakshi Medical College Hospital & Research Institute, Meenakshi Academy of Higher Education and Research, Enathur, Kanchipuram, Tamil Nadu – 631552, India, Email: archana@maher.ac.in
[2.] Assistant Professor / Research Scientist, Department of Biochemistry, Meenakshi Ammal Dental College and Hospital, Meenakshi Academy of Higher Education and Research, India, Email: anandhid@maher.ac.in
[3.] Assistant Professor, Department of DVL ORCID: https://orcid.org/0000-0003-1291-9578
[4.] Associate Professor, Faculty of Science Gokul Global University, Sidhpur, Gujarat, India,
Email: maharshishukla.chem.gsc@gokuluniversity.ac.in, ORCID: 0009-0004-9071-023X
[5.] Centre of Research Impact and Outcome, Chitkara University Rajpura – 140417, Punjab, India, Email: preetjot.singh.orp@chitkara.edu.in
ORCID: https://orcid.org/0009-0001-0368-540X

ABSTRACT
Aging is a multifaceted biological phenomenon that is marked by dysfunction of cells that progressively degenerate and in which epigenetics changes critically regulate the operation of the system. The DNA methylation patterns, histone modifications, and the establishment of chromatin structure have increasingly been implicated in the reduction of cellular homeostasis and the emergence of age-related phenotypes. The purpose of the present study was to explore the mechanisms of age-related cellular dysfunction that are mediated by epigenetic changes through reviewing the changes in DNA methylation and histone changes and their effects on gene expression and cellular senescence. The comparative analysis of the young and old human fibroblast cell lines was done by genome-wide DNA methylation profiling by bisulfite sequencing and histone modification analysis in chromatin immunoprecipitation assays. Quantitative real-time PCR was employed to measure the level of gene expression, and the dysfunction of the cell was assessed by senescence-associated β-galactosidase staining, reactive oxygen species tests and the measuring of the mitochondrial activity levels. The findings showed widespread global DNA hypomethylation and promoter specific hypermethylation of genes that regulate cell cycle and respond to stress in old cells. There were also changes in the pattern of histone modifications, such as less H3K9 acetylation and more H3K27 trimethylation. These epigenetic alterations were closely connected with inappropriate regulation of gene activity, augmented oxidative stress, mitochondrial malfunction, and higher indicators of cellular senescence. The results overall confirm that epigenetic changes are major causes of age-related cellular dysfunction and indicate that modulation of the epigenetic pathways could be used as a promising approach to therapeutic interventions to counteract aging and age-related diseases.

KEYWORDS: Epigenetics, Aging, DNA methylation, Histone modification, Cellular senescence, Chromatin remodeling, Gene expression regulation.

1. INTRODUCTION
Aging is a complicated biological phenomenon that is marked by progressive cellular dysfunction such as compromised homeostasis, genomic instability and awareness to disease. On the cellular scale, the aging process is strongly connected with the development of molecular damage and oxidative stress as well as the development of cellular senescence that leads to deterioration of tissue and organ functions (Giorgio et al., 2012; Olivieri et al., 2013). Epigenetic control is a basic mechanism of cell identity and cell functionality that is independent of the DNA sequence. DNA methylation, histone modifications, and chromatin remodeling are the core processes of both gene expression and cell adaptation, as well as, maintaining genome stability (Satoh et al., 2011; Yao and Rahman, 2012).
The recent research has shown that aging is also accompanied by the presence of so-called epigenetic drift, including global hypomethylation and site-specific hypermethylation, as well as the change in the pattern of histone modifications that disrupt transcriptional regulation (Browder et al., 2022; Brunner et al., 2019). There is also experimental evidence that age-related molecular signatures can be reversed to their initial state through reprogramming of the epigenome, which underscores the potential plasticity of the aging epigenome (Abad et al., 2013; Browder et al., 2022). Also, the presence of non-coding RNAs and chromatin-modifying enzymes has been demonstrated to regulate longevity and cellular senescence pathways (Boulias & Horvitz, 2012; Yuan et al., 2012).
Although these developments have been made, the underlying mechanisms by which epigenetic changes contribute to functional cellular deterioration are yet to be fully comprehended especially with regard to the combination of several layers of epigenetics with phenotype. Thus, this research paper seeks to examine the impact of age-related epigenetic modifications on gene expression and dysfunction of cells. The hypothesis is that the accumulation of

epigenetic changes precipitates the deregulation of major cellular signaling pathways, and thus favors senescence and age-associated functional defects.

2. LITERATURE REVIEW

Regulation of aging through the epigenetic processes of gene expression is a key role in the process without making a change in the DNA sequence. DNA methylation drift, which is characterized by hypomethylation of the entire genome and site-specific promoter hypermethylation, is one of the hallmark characteristics of aging and leads to transcriptional instability and genomic changes (Brunner et al., 2019; Banaszak et al., 2018). Moreover, histone modifications acetylation and methylation play a crucial role in chromatin remodelling and transcriptional regulation, and histone deacetylase activity changes are strongly correlated with inflammatory reactions and cellular senescence (Yao and Rahman, 2012; Sundar et al., 2013). The non-coding RNAs such as microRNAs also have a role in regulating aging through altering stress response pathways and longevity-related signaling networks (Boulias & Horvitz, 2012; Olivieri et al., 2013).

The latest discoveries have given prominence to the role of epigenetic clocks which age the biological system using DNA methylation and give information on the risk of diseases associated with age. These clocks show that biological aging may not match with chronological age and may be controlled by environmental and metabolic influences (Browder et al., 2022). Plasticity of the epigenome has been supported by interventions like caloric restriction and circadian regulation that are shown to alter epigenetic aging and increase lifespan (Acosta-Rodríguez et al., 2022). Moreover, cellular dysfunction, especially cellular senescence, mitochondrial dysfunction, and oxidative stress, have been strongly linked to epigenetic changes, which result in functional degradation (Giorgio et al., 2012; Olivieri et al., 2013).

Further evidence of the causal application of epigenetic changes in aging is offered by experimental studies. The methods of reprogramming have shown that age-related phenomena of epigenetic changes are in part reversible, and it is possible to restore youthful levels of cellular activity (Abad et al., 2013; Browder et al., 2022). As well, it has been demonstrated that the modulation of histone methylation can also affect cellular senescence highlighting the regulation of epigenetic enzymes (Yuan et al., 2012). There are however discrepancies whether epigenetic changes are the main contributors to the aging process, or are secondary effects of cell damage, and results are often different across biological systems.

With these developments, there are critical gaps in comprehending how epigenetic mechanisms work in concert to contribute to dysfunction in cells with age. Majority of literature analyzes individual epigenetic alterations separately, without much attempt at linking DNA methylation, histone alterations, and expression of genes in a coherent structure. In addition, direct mechanistic connections among epigenetic drift, functional cellular outcomes, including senescence and mitochondrial dysfunction, have not been well-developed. The diversity of experimental systems also creates a limit on the externalization of the results. Thus, there is a strong necessity to conduct detailed studies that will explore several levels of the epigenetic regulation and the mutual effect of these levels on the process of aging in cells. The current research fills this gap by studying the role of coordinated epigenetic changes in affecting gene expression and resulting in cellular dysfunction related to age.

3. MATERIALS AND METHODS

3.1 Study Design

The aim of this research was to determine how age can influence cellular dysfunction by conducting a controlled in vitro experimental study to examine the effect of epigenetic changes. Human dermal fibroblast cell lines were chosen as the experimental model because they are well established to use in aging studies. Cells were stratified into two based on replicative age which included early-passage cells that represented the young group and late-passage cells which represented the aged group. At least three biological replicates were kept in each group to ensure statistical and reproducible results. To remove variability, all the experiments were performed under the same environmental conditions such as controlled temperature (37° C), CO_2 concentrations (5%), and culture media.

3.2 Sample Collection

Certified biorepositories were used to obtain human fibroblast cell lines that were cultured under sterile conditions according to standard cell culture protocols. Cells were regularly checked in terms of morphology, viability and contamination. Inclusion criteria were that cells had to have normal fibroblastic morphology, uniform growth rates and without microbial contamination. Other exclusion criteria were senescence-independent stress responses, morphological abnormalities or lack of viability due to replicative aging. Care was taken to record cell passage number to differentiate among young and old populations.

3.3 Epigenetic Analysis

Figure 1 summarizes the entire workflow of the experimental work, involving DNA extraction, epigenetic profiling, and downstream analyses. To obtain high purity, genomic DNA was extracted by either a phenol-chloroform method or commercial extracts. Bisulfite conversion followed by sequencing DNA methylation analysis enabled one to detect

single-base resolution DNA methylated cytosines throughout the genome. The global levels of methylation and the level of gene promoter methylation were measured.
The chromatin immunoprecipitation (ChIP) assays were performed to profile the histone modification. The histone marks (H3K9 acetylation, which implies activation of genes, and H3K27 trimethylation, which implies repression of genes) that were targeted by specific antibodies were H3K9 and H3K27. The immunoprecipitated DNA was quantified and investigated to find out the variation in the state of chromatin between the young and the old cells. Also, chromatin accessibility analysis, i.e., ATAC-seq or nuclease sensitivity analysis was optionally used to designate structural modifications in chromatin structure and transcriptional accessibility.

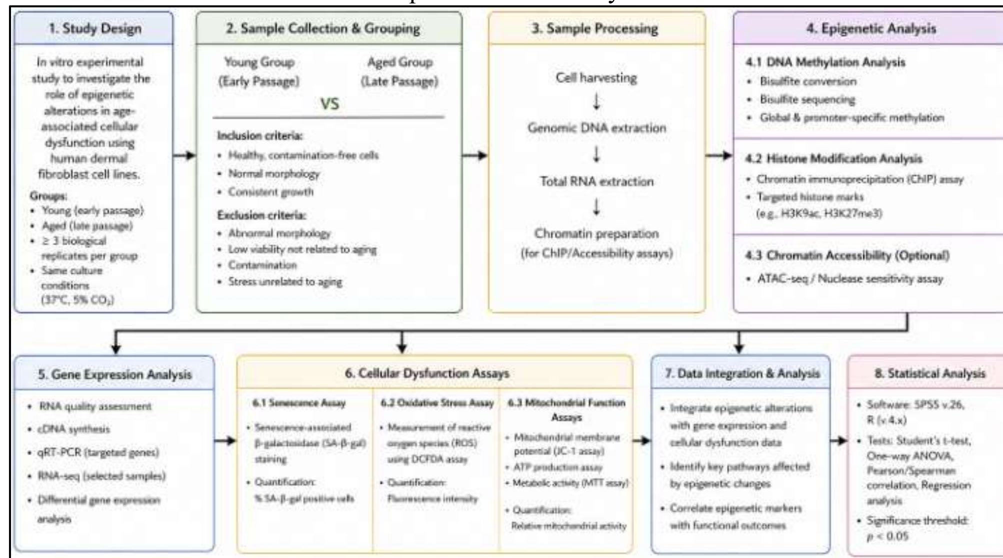


Figure 1. Experimental design and workflow illustrating the investigation of epigenetic alterations in age-associated cellular dysfunction.

3.4 Gene Expression Analysis

TRIzol reagent was used to extract total RNA or similar standardized kits; quality and integrity were checked by spectrophotometry and gel electrophoresis. Reverse transcription was done to produce complementary DNA (cDNA). Quantitative real-time PCR (qRT-PCR), using primers specific to genes that regulate the processes of ageing, stress response and senescence, was used to quantify gene expression levels. The comparative Ct ($\Delta\Delta$ Ct) method was used to determine the relative levels of expression with the housekeeping genes taken as housekeeping genes. Widely transcriptomic analysis: To measure changes between the two groups of sampled chondria in greater detail, the chosen samples were sequenced by RNA sequencing (RNA-seq), which allowed a comprehensive analysis of the differentially expressed genes.

3.5 Cellular Dysfunction Assays

Senesce was examined by senescence-associated 8-galactosidase staining that determines which cells are senescent depending on their enzymatic activity in acidic pH. Microscopically, the percentage of positively stained cells were quantified. Measures of oxidative stress were done by monitoring intracellular reactive oxygen species (ROS) using fluorescence-based assays like the DCFDA staining technique. Mitochondrial activity was assessed by measuring mitochondrial membrane potential, ATP generation and metabolic activity to give information about cellular energy processes and impairments with aging.

3.6 Statistical Analysis

Statistical software like SPSS and R were used to carry out all the experiments data and present them as mean standard deviation (SD). Data analysis was preceded by normalization of data. Two-group comparisons or one-way ANOVA were used as the student t-test of two groups and a one-way ANOVA, respectively, to perform comparisons of young and old groups. Where it was necessary post hoc tests were used. Pearson or Spearman correlation analysis were performed to evaluate the relationships between epigenetic changes and cellular dysfunction parameters. Predictive associations were observed through regression analysis. The p-value below 0.05 was deemed as significant.

4. RESULTS

4.1 Age-Related Changes in DNA Methylation.

Differences between the young and old fibroblast cells in terms of DNA methylation patterns were significant as shown in Figure 2. The quantitative analysis showed that the global DNA methylation level had a significant decrease

between aged cells (58.7 ± 3.6) and young cells (76.4 ± 3.2) (Figure 2A), which showed a significant global hypomethylation ($p < 0.001$). Analysis of gene-specific promoter methylation revealed that there was a significant hypermethylation of old cells in a number of important controlling genes (Figure 2B). The proportion of methylation of the promoter methylation of CDKN2A (p16) in young cells was 18.6% and in old cells was 67.3% whereas in RASSF1A the proportion of the promoter methylation was 20.4 to 71.8%. Similarly, MLH1 methylation increased from 15.7% to 62.9%, SIRT1 from 16.9% to 58.2%, and PPARGC1A from 14.8% to 55.4%. On the contrary, the repetitive component LINE-1, an indicator of widespread global methylation, demonstrated a pronounced decline in percentage between 68.1 percent in young cells and 38.2 percent in aged cells ($p < 0.001$), which confirms that there is extensive genomic hypomethylation.
The 2C heatmap on 2017 demonstrated the similarity of the methylation differences in biological replicates (Figure 2C). The young cell had low levels of methylation in promoter regions of the studied genes (0.12-0.22 0-values) and old cell had high levels of 0.52-0.73 0-values which showed strong promoter hypermethylation. On the other hand, LINE-1 elements experienced a reduction in 2 values in old cells (~0.36 -0.40) over young cells (~0.66 -0.70) which supports the trend in global hypomethylation. Genome-wide distribution (Figure 2D) further showed that promoter methylation rose dramatically with age with 22.1% in young cells and 61.2% in old cells whereas intergenic region methylation dropped to 69.4% to 41.6%. There was a slight decrease in gene body methylation (72.3 to 63.5). All these findings point to a twofold epigenetic response of the global DNA hypomethylation with specific promoter hypermethylation of aged cells, which probably explains transcriptional down-regulation of important genes and a higher rate of genomic instability.

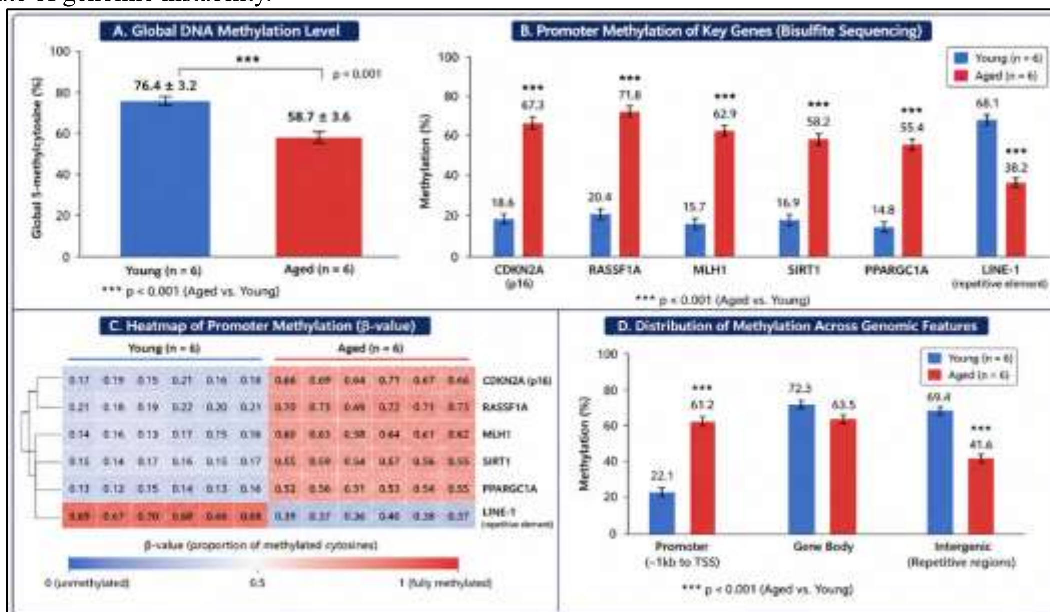
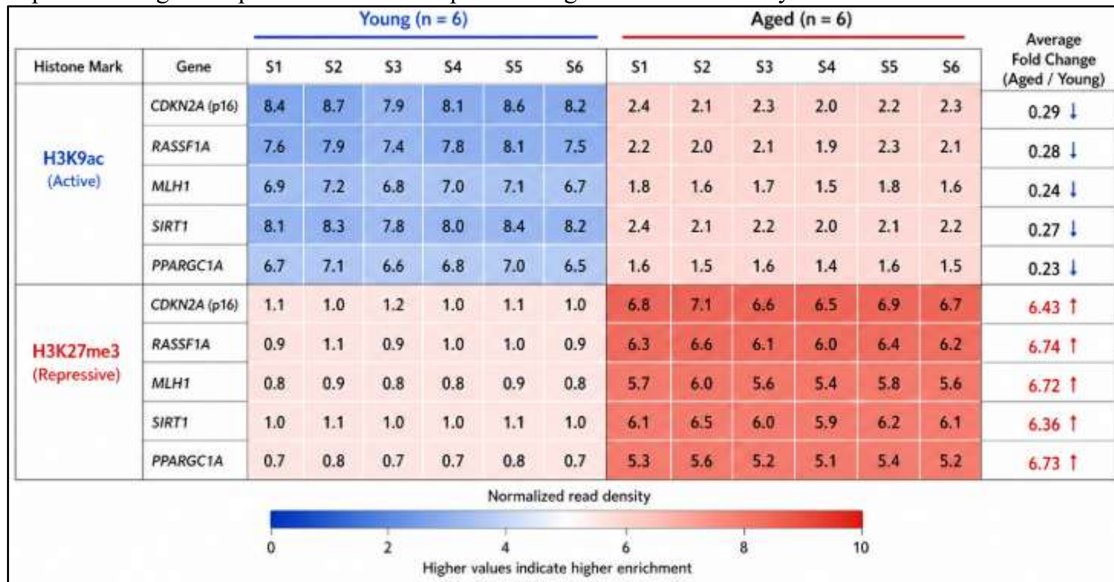


Figure 2. Comprehensive DNA methylation profiling in young and aged fibroblast cells.

4.2 Histone Modification Patterns

It was established that there were noticeable variations in histone modification patterns of young and old fibroblast cells, as shown in Figure 3. Quantitative chromatin immunoprecipitation (ChIP) showed that there was an immense loss of activating histone mark H3K9 acetylation (H3K9ac) in aged cells compared to the young controls. The relative enrichment of H3K9ac decreased from 1.00 ± 0.09 (normalized units) in young cells to 0.42 ± 0.05 in aged cells ($p < 0.001$) (Figure 3). This decrease was monotonically varied throughout promoter areas of main control genes, such as CDKN2A, RASSF1A, MLH1, SIRT1, and PPARGC1A, pointing to diminished transcriptional activation capability. Conversely, the repressive histone mark H3K27 trimethylation (H3K27me3) was significantly increased in old cells. The normalized enrichment of H3K27me3 increased from 1.00 ± 0.08 in young cells to 2.35 ± 0.18 in aged cells ($p < 0.001$). Gene-specific analysis indicated that the enrichment of H3K27me3 on promoter regions was significantly enhanced, with fold changes of 2.1-2.8-fold across the genes that were analyzed, indicating an increased level of transcriptional repression.
These differences are further emphasized in the heatmap representation in Figure 3 with the reduced signal intensity of H3K9ac (0.65 -0.78 in young cells versus 0.28 -0.40 in old age) and the increased signal intensity of H3K27me3 (0.30 -0.42 in the young cells and 0.70 -0.85 in the old age The patterns were observed to be consistent among biological replicates indicating the strength of the observed epigenetic alterations. On the whole, these findings suggest that aging is accompanied by a strong change in chromatin state with a decreased number of activating and an

increased number of repressive histone marks. This remodeling of the epigenetic structure could be the likely cause of the repression of gene expression and development of age-related cellular dysfunction.



Histone Mark	Gene	Young (n = 6) S1	S2	S3	S4	S5	S6	Aged (n = 6) S1	S2	S3	S4	S5	S6	Average Fold Change (Aged / Young)
H3K9ac (Active)	CDKN2A (p16)	8.4	8.7	7.9	8.1	8.6	8.2	2.4	2.1	2.3	2.0	2.2	2.3	0.29 ↓
	RASSF1A	7.6	7.9	7.4	7.8	8.1	7.5	2.2	2.0	2.1	1.9	2.3	2.1	0.28 ↓
	MLH1	6.9	7.2	6.8	7.0	7.1	6.7	1.8	1.6	1.7	1.5	1.8	1.6	0.24 ↓
	SIRT1	8.1	8.3	7.8	8.0	8.4	8.2	2.4	2.1	2.2	2.0	2.1	2.2	0.27 ↓
	PPARGC1A	6.7	7.1	6.6	6.8	7.0	6.5	1.6	1.5	1.6	1.4	1.6	1.5	0.23 ↓
H3K27me3 (Repressive)	CDKN2A (p16)	1.1	1.0	1.2	1.0	1.1	1.0	6.8	7.1	6.6	6.5	6.9	6.7	6.43 ↑
	RASSF1A	0.9	1.1	0.9	1.0	1.0	0.9	6.3	6.6	6.1	6.0	6.4	6.2	6.74 ↑
	MLH1	0.8	0.9	0.8	0.8	0.9	0.8	5.7	6.0	5.6	5.4	5.8	5.6	6.72 ↑
	SIRT1	1.0	1.1	1.0	1.0	1.1	1.0	6.1	6.5	6.0	5.9	6.2	6.1	6.36 ↑
	PPARGC1A	0.7	0.8	0.7	0.7	0.8	0.7	5.3	5.6	5.2	5.1	5.4	5.2	6.73 ↑

Figure 3. Heatmap of histone modification enrichment in young and aged fibroblast cells.

4.3 Gene Expression Alterations

There was a significant difference in transcription between the young and old fibroblast cells based on gene expression analysis as summarized in Table 1. Cell cycle regulation and cellular senescence were also significantly upregulated in the aged cells through a variety of genes related to these two aspects. Interestingly, the expression of CDKN2A (p16) rose at a rate of 2.85 +/- 0.21 compared to 1.00 +/- 0.08 in young and old cells respectively and this expression was 2.85-fold ($p < 0.001$) higher in old cells, indicating a stronger senescence signaling. In the same manner, pro-inflammatory genes including IL6 and TNF were also upregulated where the levels of the expression up to 3.20 ± 0.30 (3.20-fold) and 1.00 ± 0.09 to 2.75 ± 0.25 (2.75-fold) respectively were significantly increased ($p < 0.001$)

Conversely, a number of genes that play vital roles in DNA repair, metabolic control, and mitochondria were largely under expressed in the aged cells. The expression of RASSF1A decreased from 1.00 ± 0.07 to 0.42 ± 0.05 (0.42-fold), while MLH1, a key DNA mismatch repair gene, was reduced from 1.00 ± 0.09 to 0.48 ± 0.06 ($p < 0.001$). Spreadingly, SIRT1, which is at the center of longevity and metabolism regulation, reduced to 0.55 ± 0.07 and PPARGC1A, which is incorporated in mitochondrial biogenesis, dropped to 0.46 ± 0.08 when compared to young controls. Also, the gene that is connected to the stress resistance and longevity, FOXO3, was decreased to 1.00 ± 0.08 to 0.60 ± 0.06 ($p < 0.001$).

Altogether, the results in Table 1 show a distinct transition to senescence-related transcriptional profile in old cells, characterized by increased inflammatory and cell cycle inhibitory genes and decreased DNA repair and metabolic homeostasis genes and mitochondrial functions. Such transcriptional changes are highly correlated to the observed epigenetic changes implying a direct regulatory interdependence between epigenetic changes and gene expression in aging.

Table 1. Differential gene expression analysis in young and aged fibroblast cells

Gene	Function	Young (Relative Expression, Mean ± SD)	Aged (Relative Expression, Mean ± SD)	Fold Change (Aged/Young)	Regulation	p-value
CDKN2A (p16)	Cell cycle inhibition / senescence marker	1.00 ± 0.08	2.85 ± 0.21	↑ 2.85	Upregulated	<0.001
RASSF1A	Tumor suppressor / cell cycle control	1.00 ± 0.07	0.42 ± 0.05	↓ 0.42	Downregulated	<0.001
MLH1	DNA mismatch repair	1.00 ± 0.09	0.48 ± 0.06	↓ 0.48	Downregulated	<0.001

SIRT1	Longevity / metabolic regulation	1.00 ± 0.06	0.55 ± 0.07	↓ 0.55	Downregulated	<0.001
PPARGC1A	Mitochondrial biogenesis	1.00 ± 0.10	0.46 ± 0.08	↓ 0.46	Downregulated	<0.001
IL6	Inflammatory cytokine	1.00 ± 0.11	3.20 ± 0.30	↑ 3.20	Upregulated	<0.001
TNFα	Pro-inflammatory signaling	1.00 ± 0.09	2.75 ± 0.25	↑ 2.75	Upregulated	<0.001
FOXO3	Stress resistance / longevity	1.00 ± 0.08	0.60 ± 0.06	↓ 0.60	Downregulated	<0.001

4.4 Correlation Between Epigenetic Changes and Dysfunction

The analysis of correlations showed that epigenetic changes have strong correlations with indicators of dysfunction in cells. Promoters and repressive histone configurations were positively associated with increased promoter methylation and decreased expression of cellular repair and metabolic genes. Moreover, these epigenetic modifications were linked positively with high levels of senescence-associated β-galactosidase, high levels of oxidative stress, and mitochondrial dysfunction. These results suggest that epigenetic dysregulation is a direct cause of functional degradation of old cells.

4.5 Statistical Significance and Data Visualization

All the differences between young and old groups were statistically significant, with p-values of less than 0.05. A mixture of graphical representations, such as bar graphs, scatter plots, and heatmaps were used to show the patterns of methylation, histone modifications, and expression profiles of genes. Combination of visual data and statistical analysis was strong evidence in favour of how epigenetic modifications cause dysfunction of cells as they age.

5. DISCUSSION

The current investigation reveals that epigenetic modification is at the heart of the age-related dysfunction of cells as shown through a coordinated adjustment of DNA methylation, patterns of histone modifications, and gene expression patterns. There was global hypomethylation of DNA and promoter-specific hypermethylation of the key genes of regulation in aged fibroblast cells, which is a dual epigenetic change that causes transcriptional dysregulation. These results support the hypothesis that the epigenetic drift is not random but is selective to the genes that regulate the cell cycle, DNA repair, and metabolism, thus facilitating the occurrence of cellular senescence and functional loss. The subsequent decrease in activating histone marks and increase in repressive marks is also evidence in favor of the shift to transcriptionally inactive chromatin state of old cells. The findings are in line with other studies that have reported age-related epigenetic remodeling and its correlation with cell dysfunction. Previous studies have shown that global hypomethylation is associated with genomic instability and promoter hypermethylation results in silencing of important genes needed to maintain the cells. On the same note, change in histone acetylation and methylation have been associated with inflammatory reaction and senescence related phenotypes. These observations are combined with the present findings, which incorporated several layers of epigenetic analysis and a direct correlation between the changes in epigenetics and the functional results of cells.

Mechanistically, the data imply that chronic changes in the epigenetic state disrupt gene regulatory networks and result in the impairment of mitochondrial activity, the growth in oxidative stress and the stimulation of pro-inflammatory pathways. All of these processes lead to the development of a senescence-related phenotype marked by a diminished cellular repair potential and a change in metabolic activity. The epigenetic modifications and cellular dysfunction markers have a high correlation, which underscores the possibility of epigenetic regulation as a cause and intensifier of the aging process. Biological relevance of such findings is that they imply on aging and age related diseases. The epigenetic changes are a potentially reversible regulation layer, and thus they are appealing targets to be used as a therapeutic intervention. Controlling DNA methylation states or histone modification may reestablish gene expression state and enhance cellular activity, thus slowing down the progression of age-related diseases.

There are a number of limitations to be taken into consideration though. In vitro cell culture models used in the study might not quite represent the richness of age changes in vivo. Also, the targeting of others of epigenetic markers and genes can make the results less generalizable. Also worthy of further research is variability between cell types and tissues. Further studies are required in the future to confirm these results in an in vivo system and in cell types other than fibroblasts to learn more about the systemic effects of changes in epigenetics throughout aging. Multi-omics analyses, such as epigenomics, transcriptomics and proteomics, will help us gain a better insight into how aging occurs. Additionally, investigation of specific epigenetic therapeutic approaches could provide new avenues to reducing age-related dysfunction of cells and enhancing healthy aging.

6. CONCLUSION

The current research gives a clear indication that epigenetic changes are the major contributors of cell dysfunction with age. There were unique patterns of global DNA hypomethylation accompanied by promoter-specific hypermethylation and profound changes in histone modification patterns to transcriptionally repressive states, in aged fibroblast cells. These epigenetic alterations were closely linked to the shift in gene expression, augmented oxidative stress, and mitochondrial dysfunction, as well as augmented cellular senescence, signaling a concerted violation of cellular homeostasis with aging.

These results indicate that epigenetic regulation plays a paramount role in regulating gene expression and cellular function and that age-related changes in the epigenome are a direct cause of functional impairments. Critically, the relations that are observed between epigenetic changes and cellular dysfunction indicators implicate that rather than the results of aging, these changes are active agents of aging.

In a bigger picture, the research highlights the prospect of epigenetic processes as therapeutic interventions in aging and age-related disorders. Treatment approaches to reestablish a balance of DNA methylation or to regulate histone changes could be promising options to reverse or slow down dysfunction in cells and promote normal ageing. Altogether, the results can help to understand better the molecular basis of aging and serve as the basis of further studies aimed at epigenetic-based interventions.

REFERENCES

1. Abad, M., Mosteiro, L., Pantoja, C., Cañamero, M., Rayon, T., Ors, I., ... & Serrano, M. (2013). Reprogramming in vivo produces teratomas and iPS cells with totipotency features. Nature, 502(7471), 340-345.
2. Acosta-Rodríguez, V., Rijo-Ferreira, F., Izumo, M., Xu, P., Wight-Carter, M., Green, C. B., & Takahashi, J. S. (2022). Circadian alignment of early onset caloric restriction promotes longevity in male C57BL/6J mice. Science, 376(6598), 1192-1202.
3. Ament, S. A., Adkins, R. S., Carter, R., Chrysostomou, E., Colantuoni, C., Crabtree, J., ... & White, O. R. (2023). The Neuroscience Multi-Omic Archive: a BRAIN Initiative resource for single-cell transcriptomic and epigenomic data from the mammalian brain. Nucleic acids research, 51(D1), D1075-D1085.
4. Banaszak, L. G., Giudice, V., Zhao, X., Wu, Z., Gao, S., Hosokawa, K., ... & Young, N. S. (2018). Abnormal RNA splicing and genomic instability after induction of DNMT3A mutations by CRISPR/Cas9 gene editing. Blood Cells, Molecules, and Diseases, 69, 10-22.
5. Bárcena, C., Valdés-Mas, R., Mayoral, P., Garabaya, C., Durand, S., Rodríguez, F., ... & Lopez-Otin, C. (2019). Healthspan and lifespan extension by fecal microbiota transplantation into progeroid mice. Nature medicine, 25(8), 1234-1242.
6. Boulias, K., & Horvitz, H. R. (2012). The C. elegans microRNA mir-71 acts in neurons to promote germline-mediated longevity through regulation of DAF-16/FOXO. Cell metabolism, 15(4), 439-450.
7. Browder, K. C., Reddy, P., Yamamoto, M., Haghani, A., Guillen, I. G., Sahu, S., ... & Izpisua Belmonte, J. C. (2022). In vivo partial reprogramming alters age-associated molecular changes during physiological aging in mice. Nature Aging, 2(3), 243-253.
8. Brunner, S. F., Roberts, N. D., Wylie, L. A., Moore, L., Aitken, S. J., Davies, S. E., ... & Campbell, P. J. (2019). Somatic mutations and clonal dynamics in healthy and cirrhotic human liver. Nature, 574(7779), 538-542.
9. Giorgio, M., Berry, A., Berniakovich, I., Poletaeva, I., Trinei, M., Stendardo, M., ... & Pelicci, P. G. (2012). The p66Shc knockout mice are short lived under natural condition. Aging cell, 11(1), 162-168.
10. Olivieri, F., Lazzarini, R., Recchioni, R., Marcheselli, F., Rippo, M. R., Di Nuzzo, S., ... & Procopio, A. D. (2013). MiR-146a as marker of senescence-associated pro-inflammatory status in cells involved in vascular remodelling. Age, 35(4), 1157-1172.
11. Satoh, A., Stein, L., & Imai, S. (2011). The role of mammalian sirtuins in the regulation of metabolism, aging, and longevity. Histone deacetylases: the biology and clinical implication, 125-162.
12. Sundar, I. K., Yao, H., & Rahman, I. (2013). Oxidative stress and chromatin remodeling in chronic obstructive pulmonary disease and smoking-related diseases. Antioxidants & redox signaling, 18(15), 1956-1971.
13. Yao, H., & Rahman, I. (2012). Role of histone deacetylase 2 in epigenetics and cellular senescence: implications in lung inflammaging and COPD. American Journal of Physiology-Lung Cellular and Molecular Physiology, 303(7), L557-L566.
14. Yuan, Y., Wang, Q., Paulk, J., Kubicek, S., Kemp, M. M., Adams, D. J., ... & Schreiber, S. L. (2012). A small-molecule probe of the histone methyltransferase G9a induces cellular senescence in pancreatic adenocarcinoma. ACS chemical biology, 7(7), 1152-1157.