IN SILICO ANALYSIS OF NUTRIENT-SENSITIVE EPIGENETIC AGING CLOCK LOCI DURING SOMATIC CELL REPROGRAMMING TO IPSCs

Project Reference No.: *49S_MSC_0332*

College: *JAIN (Deemed-to-be University), School of Sciences, J.C. Road, Bengaluru*
Branch: *Department of Biological Sciences*
Guides: *Ms. Shweta Singh*
Dr. Kirankumar Gudagudi
Students: *Ms. Gracy Sharma*
Ms. Chandana V.
Ms. Dhwani S Kumar

Keywords:
Epigenetic clock, DNA methylation, iPSC reprogramming, Horvath CpGs, Nutrient signaling

Introduction:
Aging is a complex biological process that can be quantitatively assessed using DNA methylation-based biomarkers. The Horvath epigenetic clock, comprising specific CpG loci, is widely used to estimate biological age across tissues. During somatic cell reprogramming into induced pluripotent stem cells (iPSCs), epigenetic rejuvenation is expected to occur through resetting of age-associated methylation patterns.

Recent studies suggest that certain CpG loci are influenced by metabolic and nutrient-sensitive pathways, raising questions about whether all aging-associated loci undergo uniform resetting. Understanding the behavior of such loci during reprogramming is essential for improving regenerative medicine strategies.

This study aims to computationally analyze DNA methylation datasets to evaluate the extent of epigenetic clock resetting and identify CpG loci that may retain memory or exhibit altered behavior during reprogramming.

Objectives:

1. To analyze DNA methylation patterns at Horvath clock CpG loci.
2. To compare methylation profiles between somatic cells, iPSCs, and ESCs.
3. To classify CpG loci based on reset behavior during reprogramming.

4. To identify nutrient-sensitive CpG loci and examine their association with incomplete or altered epigenetic reset.

Methodology:

Publicly available DNA methylation datasets are obtained from the Gene Expression Omnibus (GEO) database and analyzed using R-based bioinformatics tools such as GEOquery and minfi. Data preprocessing, including normalization and filtering, is performed to ensure data quality.

Horvath epigenetic clock CpGs are extracted and matched with dataset probes. Methylation levels are then compared across Somatic, induced pluripotent stem cell (iPSC), and embryonic stem cell (ESC) groups.

Based on the relative methylation changes between these groups, CpG loci are classified into converged, partial reset, somatic memory, and overshoot categories.

Visualization techniques such as Principal Component Analysis (PCA), density plots, boxplots, and heatmaps are used to analyze methylation patterns and group-wise variation.

Result and Conclusion:

Preliminary analysis of the selected dataset indicates substantial convergence of CpG loci toward ESC-like methylation states during reprogramming. However, a subset of loci demonstrates incomplete or aberrant reset behavior, suggesting heterogeneous and locus-specific epigenetic dynamics. These findings indicate that while global epigenetic rejuvenation occurs, complete resetting may not be achieved across all CpG sites.

References

1. S. Horvath, “DNA methylation age of human tissues and cell types,” Genome Biology, vol. 14, no. 10, 2013.
2. K. Takahashi and S. Yamanaka, “Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors,” Cell, vol. 126, no. 4, pp. 663–676, 2006.
3. K. Kim et al., “Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells,” Nature Biotechnology, vol. 29, no. 12, pp. 1117–1119, 2011.
4. T. S. Mikkelsen et al., “Dissecting direct reprogramming through integrative genomic analysis,” Nature, vol. 454, pp. 49–55, 2008.

Future Scope:

1. Additional publicly available datasets will be analyzed to validate the observed epigenetic reset patterns across different conditions.
2. Pathway enrichment and gene-level analysis will be performed to identify biological processes associated with non-converging CpG loci.
3. The potential role of metabolic and nutrient-sensitive pathways in regulating epigenetic reprogramming will be further explored.
4. The computational framework developed in this study can be extended for application in aging research and regenerative medicine.