Molecular Mechanism of Stem Cells' Pluripotency

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Abstract. This study investigated the molecular mechanisms of stem cell pluripotency in Nigerian populations, addressing a critical knowledge gap in understanding population-specific variations in stem cell biology. Using comprehensive molecular analyses including transcriptomics, epigenomics, and functional characterisation, we examined induced pluripotent stem cells (iPSCs) derived from diverse Nigerian ethnic groups.

While core pluripotency transcription factors (OCT4, SOX2, NANOG) showed strong conservation, Nigerian-derived iPSCs exhibited distinctive molecular features. Specifically, these cells demonstrated altered isoform usage, population-specific expression patterns in extended pluripotency networks, and unique epigenetic landscapes.

Signalling pathway analysis revealed enhanced WNT/β-catenin activity, heightened JAK/STAT sensitivity, and reduced FGF/ERK signalling compared to reference lines. Novel regulatory elements were identified, including population-specific enhancers, long non-coding RNAs, alternative promoters, and chromatin interaction hubs.

Functional characterisation demonstrated that Nigerian-derived iPSCs possess enhanced stress resistance and distinctive differentiation propensities, with significantly higher efficiency in hematopoietic lineage specification. The cells demonstrated approximately 30% better viability under challenging conditions and a 25% improvement in hematopoietic progenitor generation compared to the reference lines.

Comparative analysis positioned Nigerian-derived iPSCs as molecularly distinct from European and East Asian lines while sharing features with other African-derived cells. These findings challenge the concept of a universal pluripotent state, supporting a model of population-specific implementations of pluripotency that achieve similar functional outcomes through different molecular configurations.

Keywords: Stem cell pluripotency; induced pluripotent stem cells; Nigerian population; epigenetic regulation; transcription factors; signalling pathways; regenerative medicine; population-specific features; molecular mechanisms; genetic diversity.

INTRODUCTION

Stem cells represent a transformative frontier in biomedical research, offering unprecedented opportunities for understanding human development, disease mechanisms, and therapeutic interventions. These remarkable cells possess two fundamental characteristics that distinguish them from other cell types: the capacity for self-renewal and the ability to differentiate into specialised cell lineages. The molecular mechanisms underpinning pluripotency – the ability to develop into any cell type in the adult organism – remain at the forefront of scientific inquiry, with

significant implications for regenerative medicine and personalised therapeutic approaches.

The journey of stem cell research has been marked by significant discoveries, gaining substantial momentum following the successful isolation of human embryonic stem cells and the revolutionary development of induced pluripotent stem cells (iPSCs). Despite these advances, a critical knowledge gap persists in understanding the molecular intricacies of stem cell pluripotency across diverse populations, particularly in African contexts.

In the Nigerian context, stem cell research presents both extraordinary opportunities and unique challenges. As Africa's most populous nation with a diverse genetic landscape, Nigeria stands to gain significantly from advances in stem cell technology. However, molecular investigations into stem cell pluripotency in Nigerian populations remain remarkably limited, despite the potential for uncovering population-specific regulatory mechanisms that could inform the development of tailored therapeutic strategies.

Several critical challenges complicate the research landscape. Existing stem cell research has predominantly focused on cell lines derived from European and Asian populations, creating a significant blind spot in understanding population-specific variations. Genetic diversity influences cellular responses to environmental factors and drug treatments, suggesting that population-specific variations in pluripotency mechanisms likely exist but remain unexplored in the Nigerian context.

Moreover, the interplay between environmental factors specific to the Nigerian setting and the molecular regulation of pluripotency remains poorly understood. Factors such as endemic diseases, nutritional status, and exposure to region-specific environmental conditions may profoundly influence epigenetic programming and stem cell behaviour. Technological limitations facing Nigerian researchers, including limited access to advanced molecular techniques, further compound these knowledge gaps.

This *research aims* to address these critical limitations by conducting a comprehensive investigation into the molecular mechanisms underlying stem cell pluripotency using samples derived from Nigerian subjects. By elucidating population-specific variations in pluripotency networks, the study seeks to contribute to the development of more effective stem cell-based therapies tailored to the Nigerian population while establishing a foundation for further stem cell research in the West African context.

The primary objectives include characterising the expression profiles of pluripotency markers, mapping regulatory networks, identifying epigenetic landscapes, investigating population-specific variations, and developing computational models that integrate transcriptomic, epigenomic, and proteomic data to predict regulatory relationships within the pluripotency network.

Achieving these objectives will substantially advance our understanding of stem cell biology in the Nigerian context while contributing to the broader field of regenerative medicine. The findings will inform the development of more effective protocols for deriving, maintaining, and directing the differentiation of stem cells from Nigerian populations, potentially leading to improved cellular therapies for conditions prevalent in West Africa.

By systematically investigating the molecular mechanisms of stem cell pluripotency in Nigerian populations, this research addresses a critical gap in global stem cell understanding. It promises to reveal how genetic background influences cellular plasticity, potentially uncovering novel regulatory mechanisms with broader implications for developmental biology, personalised medicine, and our comprehension of human cellular adaptability.

METHOD

The study employed a mixed-methods experimental research design integrating laboratory-based molecular analyses with computational modelling to comprehensively characterise the molecular mechanisms underlying stem cell pluripotency in Nigerian populations. The approach followed a sequential explanatory strategy, where initial experimental findings informed subsequent computational analyses, creating an iterative process that strengthens data interpretation and hypothesis refinement.

Sample Collection and Ethical Considerations. Ethical approval was obtained from the institutional review boards of the participating Nigerian institutions, including the Lagos University Teaching Hospital, the University of Nigeria Teaching Hospital, and the Ahmadu Bello University Teaching

Hospital. The National Health Research Ethics Committee of Nigeria provided overarching approval for the multicentre research.

Participant recruitment employed purposive sampling, targeting adult volunteers aged 18-35 years who did not have chronic health conditions. Three types of biological samples were collected: dermal fibroblasts from skin punch biopsies, peripheral blood mononuclear cells, and exfoliated deciduous teeth. The sample collection process incorporated comprehensive informed consent procedures, including multi-stage engagement, group information sessions, and individual consultations. Materials were translated into multiple local languages to ensure comprehensive understanding.

Cell Culture and Reprogramming. Primary cell isolation utilised enzymatic digestion techniques optimised for Nigerian sample characteristics. Induced pluripotent stem cells (iPSCs) were generated using non-integrating episomal vectors encoding pluripotency factors. The reprogramming protocol was modified to enhance efficiency for African population samples, incorporating specific stress protection mechanisms.

Emerging iPSC colonies underwent rigorous quality control, including morphological assessment, alkaline phosphatase staining, immunocytochemistry for pluripotency markers, karyotype verification, and demonstration of trilineage differentiation potential. Cryopreservation protocols were adapted to accommodate challenging infrastructure conditions, ensuring high cell recovery rates.

Molecular Characterisation Techniques

Transcriptomic Analysis. RNA sequencing was performed using the Illumina NovaSeq 6000 platform, with paired-end reads of 150 bp. The analysis approach incorporated comprehensive preprocessing, including adapter trimming, quality filtering, and removal of ribosomal RNA sequences. Quantitative RT-PCR provided validation of key RNA-seq findings.

Single-cell RNA sequencing complemented bulk transcriptomics, utilising the 10x Genomics Chromium system to reveal cellular heterogeneity within pluripotent stem cell populations. The protocol was optimised to improve single-cell yield while maintaining high viability.

Proteomic analysis employed a fractionation approach to separately isolate cytoplasmic and nuclear proteins, enabling the comprehensive characterisation of pluripotency-associated transcription factors. Western blotting utilised a fluorescence-based detection system for improved quantitative accuracy, with specialised conditions incorporated to examine post-translational modifications.

Epigenetic Profiling. Comprehensive epigenetic analyses included whole-genome bisulfite sequencing for DNA methylation, chromatin immunoprecipitation sequencing for histone modifications, and ATAC-seq for chromatin accessibility. These techniques enabled detailed mapping of the epigenetic landscape associated with pluripotency.

Advanced Molecular Techniques. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) mapped protein-DNA interactions, with optimised protocols developed specifically for pluripotent stem cells. CUT&RUN technology provided high-resolution mapping of protein binding sites with reduced background noise.

Computational Analysis. Bioinformatics analyses integrate diverse molecular datasets through sophisticated computational approaches. RNA-seq data analysis incorporated advanced statistical methods to identify differential expression, while network analysis reconstructed regulatory circuits using both prior knowledge and data-driven approaches.

The computational workflow included careful batch correction to mitigate technical variations between studies, enabling reliable comparisons across different datasets. Specialised adaptations were implemented to optimise analysis on moderate-performance computing systems typical in resource-limited settings.

Statistical Approaches. Statistical analysis balanced rigour with the practical constraints of limited sample sizes. Techniques included negative binomial generalised linear models for transcriptomic data, empirical Bayes methods for ChIP-seq analysis, and non-parametric approaches for protein-level quantification. Power calculations guided sample size determination, ensuring robust detection of population-specific variations.

This comprehensive methodology integrates cutting-edge molecular techniques with computational analyses, specifically adapted to the Nigerian research context. The approach enabled unprecedented molecular characterisation of stem

cell pluripotency in Nigerian populations, addressing critical knowledge gaps while maintaining scientific rigour.

RESULTS AND DISCUSSION

Demographic Characteristics of Study Participants. The study comprised 45 participants, with successful induced pluripotent stem cell (iPSC) derivation achieved in 38 individuals, resulting in an 84.4% success rate. The participant cohort ranged in age from 18 to 35 years, with a nearly balanced gender distribution of 22 females (48.9%) and 23 males (51.1%).

Ethnic representation in Nigeria reflected the country's major population groups: 28.9% Yoruba, 24.4% Igbo, 20% Hausa/Fulani, and 26.7% from other ethnic groups, including Ijaw, Kanuri, Ibibio, Tiv, and Edo. The geographical distribution across the southwestern, southeastern, and northern regions ensured comprehensive representation of genetic diversity.

Cell source diversification revealed varying reprogramming efficiencies: dermal fibroblasts showed the highest success rate at 90%, peripheral blood mononuclear cells at 80%, and dental pulp stem cells also at 80%. Notably, 15.6% of participants reported a family history of sickle cell disease, a characteristic consistent with the population's genetic profile.

Pluripotency Marker Expression Profiles. Core pluripotency transcription factors OCT4, SOX2, and NANOG demonstrated consistent expression across all Nigerian-derived lines. However, subtle variations emerged in transcript characteristics. The OCT4B isoform showed approximately 15% higher expression relative to OCT4A compared to reference lines, suggesting population-specific splicing regulation.

Extended pluripotency network genes revealed more pronounced population-specific expression patterns. DPPA4, DNMT3L, and PRDM14 exhibited significantly higher expression, while GDF3, LEFTY1, and ZFP42 displayed reduced expression in Nigerian-derived lines. These differences suggest nuanced variations in the stabilisation of pluripotency networks.

Non-coding RNAs demonstrated particularly striking population-specific expression. Long non-coding RNAs LINC00458 and LINC-ROR showed substantially higher expression, while LINC00673 expression was lower compared to the reference lines. These variations may help fine-tune the regulation of pluripotency networks.

Regulatory Network Characteristics. The core autoregulatory circuit, comprising OCT4, SOX2, and NANOG, demonstrated remarkable conservation, with over 85% of binding sites overlapping between the Nigerian-derived and reference lines. However, 783 population-specific OCT4 binding sites, 621 population-specific SOX2 binding sites, and 893 population-specific NANOG binding sites were identified.

Network modelling revealed 23 gene expression modules, with three smaller modules showing significant differences in connectivity patterns between population groups. These modules were enriched for genes involved in metabolic regulation, innate immunity, and extracellular matrix organisation, suggesting population-specific network adaptations.

Signalling Pathway Dynamics. Signalling pathway analysis uncovered distinctive characteristics in Nigerian-derived iPSCs. The WNT/β-catenin pathway exhibited enhanced activity, with approximately 1.7-fold higher levels of active β-catenin compared to reference lines. Conversely, the FGF/ERK pathway demonstrated significantly lower basal activity, with a 40% reduction in phosphorylated ERK1/2.

The TGF-β/Activin/Nodal pathway revealed nuanced population-specific dynamics. Nigerian-derived iPSCs demonstrated more rapid pathway activation, reaching maximal phosphorylation at 30 minutes compared to 60 minutes in reference lines, with faster deactivation associated with higher expression of negative feedback regulators.

The JAK/STAT pathway showed enhanced sensitivity, with LIF stimulation inducing STAT3 phosphorylation at concentrations approximately half those required for reference lines. This heightened responsiveness correlated with higher expression of pathway components GP130 and JAK1.

Epigenetic Landscape. DNA methylation analysis revealed a characteristic pattern of hypomethylation associated with pluripotency, with subtle local differences. 3,827 differentially methylated regions were identified, with significant enrichment in regulatory regions associated with developmental and immune response genes.

Histone modification profiling maintained the characteristic bivalent chromatin domains, with 14,253 domains identified in Nigerian-derived iPSCs. Population-specific bivalent domains showed enrichment for genes involved in hematopoietic and neural crest differentiation programs.

Functional Characteristics. Nigerian-derived iPSCs demonstrated enhanced stress resistance, maintaining approximately 30% better viability under challenging conditions. Differentiation propensities showed distinctive patterns, with 25% higher efficiency in generating hematopoietic progenitors but delayed kinetics in neuroectodermal differentiation.

Comparative Analysis. Global dataset comparisons positioned Nigerian-derived iPSCs as molecularly distinct from European and East Asian lines while showing similarities to other African-derived lines. A core set of 128 genes consistently distinguished African-derived induced pluripotent stem cells (iPSCs), defining an African-specific pluripotency signature enriched in genes related to stress response, metabolic regulation, and specific signalling pathways.

These comprehensive results provide a nuanced understanding of stem cell pluripotency in Nigerian populations, revealing both conserved fundamental mechanisms and population-specific molecular adaptations that reflect the unique genetic heritage of these cell lines.

Molecular Signatures of Nigerian Stem Cell Populations. The molecular characterisation of Nigerian-derived induced pluripotent stem cells (iPSCs) reveals a complex landscape of conserved pluripotency features and population-specific signatures that challenge existing paradigms of stem cell biology. While the core pluripotency transcription factor network demonstrated remarkable conservation, subtle variations emerged that provide profound insights into how genetic background influences cellular regulation.

The conservation of the fundamental regulatory roles of OCT4, SOX2, and NANOG underscores the universal nature of the pluripotency machinery across human populations. However, the observed enrichment of the OCT4B isoform in Nigerian-derived lines suggests a nuanced fine-tuning of core regulatory components. Previous studies have associated the OCT4B variant with enhanced stress resistance, potentially reflecting adaptive molecular configurations shaped by population-specific environmental pressures.

Table 1 – Comparative Expression of Pluripotency Network Genes in Nigerian-derived vs Reference iPSCs

Gene	Nigerian-derived iPSCs	Reference iPSCs	Fold Change	Significance
DPPA4	High Expression	Baseline	1.7	p<0.01
DNMT3L	Elevated	Standard	2.1	p<0.005
PRDM14	Increased	Reference	1.6	p<0.01
GDF3	Reduced	Standard	0.68	p<0.05
LEFTY1	Lower	Baseline	0.72	p<0.05
ZFP42	Diminished	Reference	0.83	p<0.05

Extended pluripotency network genes revealed more pronounced population-specific expression patterns, highlighting the dynamic nature of stem cell regulatory networks. The elevated expression of DPPA4 and PRDM14 is particularly intriguing, as these factors play crucial roles in maintaining pluripotency and facilitating epigenetic resetting. These variations suggest that population-specific molecular configurations can achieve similar functional outcomes through distinctively adapted regulatory strategies.

Signalling and Epigenetic Landscape. The distinctive signalling pathway characteristics observed in Nigerian-derived iPSCs provide compelling evidence of population-specific cellular adaptation. Enhanced WNT/β-catenin and JAK/STAT signalling, coupled with reduced FGF/ERK activity, creates a unique molecular environment that potentially reflects historical selective pressures. This signalling configuration may contribute to enhanced cellular resilience and metabolic flexibility, characteristics that could represent adaptive responses to specific environmental challenges.

Epigenetic analysis revealed a sophisticated interplay between genetic background and regulatory mechanisms. The identification of population-specific differentially methylated regions, particularly those enriched near developmental and immune response genes, suggests that epigenetic programming reflects population-specific genetic and environmental histories. The correlation between these epigenetic variations and genetic variants with population-specific allele frequencies highlights the intricate relationship between genetic sequence and functional regulation.

Table 2 – Signalling Pathway Characteristics in Nigerian-derived iPSCs

Pathway	Nigerian-derived iPSCs	Reference iPSCs	Key Variations
WNT/β-catenin	Enhanced (1.7× activity)	Standard	Increased basal activity
FGF/ERK	Reduced (0.6× p-ERK)	Reference	Lower phosphorylation
TGF-β/Activin	Rapid activation	Slower response	30 min vs 60 min peak
JAK/STAT	Heightened sensitivity	Standard	2× LIF response

Functional and Translational Implications. The enhanced stress resistance demonstrated by Nigerian-derived iPSCs represents a particularly significant finding with broad implications. The cells' ability to maintain approximately 30% better viability under challenging conditions suggests that molecular adaptations may be crucial in resource-limited research and clinical settings. This resilience may reflect deeper evolutionary adaptations to variable environmental conditions.

Differentiation propensities showed distinctive characteristics, with notably enhanced hematopoietic lineage specification. This finding has particular relevance to conditions prevalent in Nigerian populations, such as sickle cell disease. The ability to generate hematopoietic progenitors with 25% higher efficiency opens promising avenues for developing targeted cellular therapies.

Broader Scientific Significance. The research challenges the concept of a universal pluripotent state, instead supporting a model of pluripotency as a cellular condition with population-specific implementations. This perspective has profound implications for stem cell research, suggesting that a comprehensive understanding requires studying diverse genetic backgrounds.

The identified population-specific molecular features likely represent a complex interplay of neutral genetic variations and adaptive responses to selective pressures. The enrichment of variations in stress response, immune function, and metabolic regulation pathways indicates that these systems have experienced distinct evolutionary pressures across human populations.

Regenerative Medicine Considerations. From a practical standpoint, the findings provide a foundation for optimising stem cell protocols specifically for Nigerian populations. The population-specific signalling pathway characteristics and differentiation propensities inform rational approaches to derive, maintain, and differentiate stem cells with enhanced efficiency and relevance.

The research demonstrates that studying stem cell biology in diverse populations serves dual purposes: advancing fundamental scientific understanding and enabling the development of population-appropriate stem cell technologies. The molecular insights gained can potentially improve the effectiveness and accessibility of regenerative medicine approaches.

Limitations and Future Perspectives. While the study offers unprecedented insights, it also has limitations. The sample, though comprehensive, represents only a portion of Nigeria's extensive genetic diversity. Future research should expand sampling to include additional ethnic groups and conduct more extensive comparative analyses across African populations.

Ultimately, this research sheds light on the remarkable molecular complexity of human stem cell biology. It underscores the importance of diversity in scientific research, demonstrating how studying populations previously underrepresented in scientific literature can unveil novel insights with potentially transformative implications for our understanding of cellular biology and human adaptation.

CONCLUSIONS

The comprehensive investigation into the molecular mechanisms of stem cell pluripotency in Nigerian populations has yielded groundbreaking insights that challenge and expand our understanding of cellular plasticity across diverse genetic backgrounds. This research has demonstrated that while pluripotency maintains a universally conserved core mechanism, significant population-specific features exist that reflect complex genetic and evolutionary adaptations.

The study revealed that core pluripotency transcription factors OCT4, SOX2, and NANOG maintain their fundamental regulatory roles, yet exhibit subtle variations in Nigerian-derived induced pluripotent stem cells (iPSCs). Most notably, the enrichment of the OCT4B isoform suggests that population-specific fine-tuning of even the most fundamental regulatory components may be occurring, potentially reflecting adaptive

molecular configurations shaped by unique environmental pressures.

Signalling pathway dynamics emerged as a critical distinguishing feature, with Nigerian-derived iPSCs demonstrating enhanced WNT/β-catenin and JAK/STAT signalling alongside reduced FGF/ERK activity. These distinctive pathway characteristics create a unique molecular environment that may contribute to enhanced cellular resilience and metabolic flexibility, potentially representing evolutionary adaptations to specific environmental challenges.

The epigenetic landscape revealed a sophisticated interplay between genetic background and regulatory mechanisms. Population-specific differentially methylated regions, particularly those associated with genes involved in developmental and immune responses, highlight the intricate relationship between genetic sequence and functional regulation. This finding highlights the importance of studying stem cell biology across diverse populations to gain a comprehensive understanding of cellular adaptability.

Functionally, Nigerian-derived iPSCs demonstrated remarkable characteristics, including enhanced stress resistance and distinctive differentiation propensities. The ability to maintain approximately 30% better viability under challenging conditions and generate hematopoietic progenitors with 25% higher efficiency represents a significant advance with potential implications for regenerative medicine in resource-limited settings.

The research challenges the concept of a universal pluripotent state, instead supporting a model of pluripotency as a cellular condition with population-specific implementations. This perspective fundamentally transforms our understanding of stem cell biology, emphasising that diverse genetic backgrounds can achieve similar functional outcomes through distinctively adapted molecular configurations.

From a practical standpoint, the findings provide a foundation for optimising stem cell protocols specifically for Nigerian populations. The population-specific molecular signatures inform rational approaches to derive, maintain, and differentiate stem cells with enhanced efficiency and relevance, potentially revolutionising regenerative medicine strategies in the Nigerian context.

The discovery of novel regulatory elements, including population-specific enhancers, long non-coding RNAs, and distinctive chromatin interaction hubs, expands the known repertoire of pluripotency regulation. These findings underscore the crucial importance of studying stem cell biology in diverse populations, highlighting how genetic diversity can reveal regulatory mechanisms that may be obscured when research focuses on limited genetic backgrounds.

Ultimately, this research represents a significant stride in understanding human cellular biology. It illustrates how studying populations previously underrepresented in scientific literature can unveil transformative insights, bridging critical knowledge gaps and opening new avenues for personalised regenerative medicine. The findings not only advance scientific understanding but also provide a framework for developing more inclusive and effective stem cell technologies that can benefit diverse global populations.

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