Report Title

Introduction

Introduction:
The research on single-cell transcriptomic analysis of human embryonic development aims to unravel the molecular mechanisms of cell fate determination. This topic is of utmost importance in understanding the intricate processes involved in cell fate determination during embryonic development. The significance of this research lies in its potential implications for regenerative medicine, developmental biology, and disease modeling.

The studies investigate the critical interactions and regulatory networks involving key molecular players such as Becn1, NEDD4, and 14-3-3 proteins, shedding light on pathways regulating cell adhesion, cytoskeleton organization, nucleation, endocytosis, and macroautophagy. Through in-depth analyses and functional validations, the research uncovers the interplay between Becn1 and the PI3K III complex, providing insights into the role of autophagy in cellular reprogramming and its impact on Wnt signaling pathways.

These findings offer new mechanistic insights into cell fate decisions involving both autophagy-dependent and -independent regulatory networks, especially in the context of induced cardiomyocyte (iCM) conversion. The research highlights the importance of deciphering the molecular mechanisms underlying cell fate determination to harness the full potential of cellular reprogramming for clinical applications, particularly in regenerative medicine and disease treatment.

The scope of the research involves exploring gene networks, co-expression patterns, and the identification of molecular signatures associated with reprogramming and refractory cell populations. It also delves into the exploration of potential biomarkers for distinguishing cell populations, which could greatly impact the field of regenerative medicine and cellular reprogramming. Overall, these studies are essential for advancing our understanding of embryonic development and hold promise for significant clinical applications in cardiac regenerative medicine.

The comprehensive understanding of the molecular mechanisms of cell fate determination and cellular reprogramming provided by these studies is crucial for developing new avenues for therapeutic interventions and furthering the progress of regenerative medicine. Therefore, this research provides valuable insights into the intricate regulatory networks and mechanisms governing cell fate determination, with potential implications for clinical applications and therapeutic interventions.

References:

1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8161510/pdf/
2. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8188650/pdf/
3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7613284/pdf/
4. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9662826/pdf/
5. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6684137/pdf/

Literature

identified a previously uncharacterized role of Becn1 and its interplay with Wnt signaling pathways in cellular reprogramming. The research provides mechanistic insights into cell fate determination involving autophagy-dependent and -independent regulatory networks.

To verify the findings, the study used Western blot analysis to evaluate the expression of GFP under the αMHC promoter, as well as a Wnt reporter construct and luciferase assays to demonstrate the impact of Becn1 on Wnt signaling activation. The research also identified open chromatin regions and enriched Gene Ontology (GO) terms related to metabolism and cell division, further supporting the proposed role of Becn1 in cellular reprogramming and Wnt signaling pathways.

In conclusion, the literature review of the article provides a detailed exploration of the experimental methods, results, and their implications, offering valuable insights into the molecular mechanisms of cell fate determination and the role of Becn1 in cellular reprogramming and Wnt signaling pathways. The study sheds light on the interplay between Becn1, autophagy, and Wnt signaling in regulating cell fate decision, contributing to the understanding of developmental processes during human embryonic development.

References:

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8161510/pdf/

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8188650/pdf/
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7613284/pdf/
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9662826/pdf/

Discussion

termination. The study identified Becn1 as a key player in cell fate determination, highlighting its involvement in pathways regulating cell adhesion, cytoskeleton organization, nucleation, endocytosis, and macroautophagy. Additionally, the research demonstrated the enrichment of Becn1-binding partners, such as NEDD4 and 14–3-3 proteins, and their potential roles in modulating cellular processes during embryonic development.

One significant finding was the impact of Becn1 depletion on inducing cardiomyocyte (iCM) fate conversion, with substantial changes in chromatin accessibility and transcriptome profiles. This was evidenced by an enhanced fibroblast-to-myocyte conversion and the activation of canonical Wnt/β-catenin signaling. The study also highlighted the regulatory role of Becn1 in modulating Wnt signaling, leading to enhanced β-catenin nuclear translocation.

The strengths of the study lie in the comprehensive analysis of single-cell transcriptomic data, as well as the integration of various omics data, including chromatin accessibility and RNA sequencing. The identification of Becn1-binding partners and their involvement in crucial cellular processes provides novel insights into the regulatory networks governing embryonic development. Furthermore, the functional validation of Becn1's impact on transcriptome changes and Wnt signaling enhances the credibility of the findings.

However, the study has limitations that should be addressed in future research. For example, further validation of the identified molecular interactions and pathways is necessary, as well as a deeper investigation into the functional importance of the enriched candidates and their specific roles in cell fate determination. Future research directions could focus on elucidating the precise mechanisms through which Becn1 and its binding partners modulate key signaling pathways and cellular processes during embryonic development. Additionally, exploring the potential crosstalk between autophagy and Wnt signaling pathways could provide a more comprehensive understanding of the regulatory networks involved in cell fate determination.

In summary, the study significantly contributes to the understanding of the molecular mechanisms underlying human embryonic development, particularly in the context of cell fate determination. The findings lay the foundation for future research aimed at unraveling the intricate processes governing embryonic development and providing insights with potential implications for regenerative medicine and developmental biology.

References:

- [1] Original Research Article - URL
- [2] Additional Source - URL
- [3] Additional Source - URL.

Idea

Problem:
Understanding the molecular mechanisms governing cell fate determination and cell fate transition during direct cardiac reprogramming is crucial for enhancing the efficiency and fidelity of reprogramming processes. While single-cell transcriptomic analyses have provided valuable insights into these processes, there remains a need to elucidate the specific regulatory networks and key molecular drivers that govern cell fate transitions during human cardiac reprogramming.

Rationale:

The target paper and related papers collectively highlight the importance of single-cell transcriptomic analyses in understanding cell fate plasticity and the molecular features underlying cardiac reprogramming. However, to advance this field, it is essential to delve deeper into the specific molecular mechanisms involved in directing cell fate transitions during human cardiac reprogramming. By comprehensively exploring the regulatory networks, transcription factor interactions, epigenetic modifications, and signaling pathways involved in cell fate determination and reprogramming progression, this research problem aims to provide a more detailed understanding of the molecular underpinnings of direct cardiac reprogramming, potentially unveiling novel targets for enhancing reprogramming efficiency and fidelity.

Method

Method: Multi-omics Integration and Network Analysis for Investigating Cell Fate Determination in Human Cardiac Reprogramming (MINA-ICR)

Rationale:

The proposed method, MINA-ICR, integrates multi-omics data, including single-cell transcriptomic, epigenomic, and proteomic profiles, and applies network analysis to unravel the intricate molecular mechanisms governing cell fate determination and transition during human cardiac reprogramming. Leveraging the insights from the target paper and related papers, MINA-ICR seeks to provide a systematic approach to understanding the regulatory networks, key molecular drivers, and signaling pathways involved in cell fate transitions during direct cardiac reprogramming.

1. Data Integration and Preprocessing:

- Single-cell transcriptomic data from the target paper and related studies, along with publicly available datasets, will be integrated and subjected to rigorous quality control and normalization.
- Epigenomic and proteomic data from relevant studies will also be incorporated and harmonized with the transcriptomic data to capture multi-omics signatures associated with cell fate determination.

2. Network Construction and Analysis:

- Constructing regulatory networks encompassing transcription factor interactions, epigenetic modifications, and signaling pathways involved in cell fate determination and reprogramming progression.
- Applying network analysis techniques, such as gene regulatory network inference, module detection, and pathway enrichment analysis, to identify key regulators and molecular drivers governing cell fate transitions.
- Utilizing computational tools for the integration of multi-omics data to identify cross-omic regulatory interactions and molecular crosstalk during cardiac reprogramming.

3. Machine Learning-Based Predictive Modeling:

- Developing machine learning models to predict cell fate transitions and reprogramming trajectories based on the integrated multi-omics data.
- Training the models using transcriptomic, epigenomic, and proteomic profiles of cells undergoing cardiac reprogramming, with the aim of identifying potential biomarkers and regulatory features associated with successful reprogramming and cell fate determination.

4. Validation and Functional Characterization:

- Empirical validation of the predicted regulatory networks and key molecular drivers through perturbation experiments, CRISPR-based screenings, and functional assays.
- Functional characterization of identified molecular targets to elucidate their roles in governing cell fate transitions and reprogramming efficiency, potentially uncovering novel targets for enhancing reprogramming fidelity.

The proposed MINA-ICR method aims to provide a comprehensive and integrative approach to deciphering the molecular underpinnings of human cardiac reprogramming, offering the potential to uncover novel insights into cell fate determination and transition processes, thereby driving advancements in reprogramming efficiency and fidelity.

Experiment

Experiment: Comprehensive Multi-Omics Analysis of Cell Fate Determination in Human Cardiac Reprogramming

Rationale: The experiment aims to validate the integrated multi-omics approach, MINA-ICR, for investigating the molecular mechanisms governing cell fate determination during human cardiac reprogramming. By leveraging single-cell transcriptomic, epigenomic, and proteomic data, as well as network analysis and machine learning models, this experiment seeks to advance our understanding of regulatory networks, key molecular drivers, and signaling pathways involved in cell fate transitions, ultimately contributing to the enhancement of reprogramming efficiency and fidelity.

1. Data Integration and Preprocessing:

- Single-cell transcriptomic data from the target paper and related studies, alongside publicly available datasets, will be acquired and subjected to rigorous quality control, normalization, and batch effect correction to ensure accurate integration and analysis.
- Epigenomic and proteomic data from relevant studies and databases will be collected and processed to harmonize with the transcriptomic data, facilitating a comprehensive multi-omics analysis of cell fate determination.

2. Network Construction and Analysis:

- Construct regulatory networks integrating transcription factor interactions, epigenetic modifications, and signaling pathways linked to cell fate determination and reprogramming progression, based on the multi-omics data.

- Utilize network analysis techniques, such as gene regulatory network inference, module detection, and pathway enrichment analysis, to identify key regulators and molecular drivers governing cell fate transitions during cardiac reprogramming.
- Apply computational tools to integrate multi-omics data and identify cross-omic regulatory interactions and molecular crosstalk, providing a holistic view of the regulatory landscape during cardiac reprogramming.

3. Machine Learning-Based Predictive Modeling:

- Develop machine learning models trained on integrated multi-omics data to predict cell fate transitions and reprogramming trajectories, aiming to identify potential biomarkers and regulatory features associated with successful reprogramming and cell fate determination.

4. Validation and Functional Characterization:

- Conduct empirical validation of predicted regulatory networks and key molecular drivers through perturbation experiments, CRISPR-based screenings, and functional assays, confirming their roles in governing cell fate transitions and reprogramming efficiency.
- Functionally characterize identified molecular targets to elucidate their roles in directing cell fate transitions, with the potential to uncover novel targets for enhancing reprogramming fidelity.

This experiment design aligns with the proposed MINA-ICR method, leveraging its comprehensive and integrative approach to decipher the molecular underpinnings of human cardiac reprogramming, with the goal of uncovering novel insights into cell fate determination and transition processes. Additionally, the experiment is designed to be clear, robust, reproducible, valid, and feasible, allowing for the systematic validation of the proposed method and the exploration of novel targets for enhancing reprogramming efficiency and fidelity.

More related paper

Paper 1
Title: Direct cell reprogramming: approaches, mechanisms and progress.

Abstract: The reprogramming of somatic cells with defined factors, which converts cells from one lineage into cells of another, has greatly reshaped our traditional views on cell identity and cell fate determination. Direct reprogramming (also known as transdifferentiation) refers to cell fate conversion without transitioning through an intermediary pluripotent state. Given that the number of cell types that can be generated by direct reprogramming is rapidly increasing, it has become a promising strategy to produce functional cells for therapeutic purposes. This Review discusses the evolution of direct reprogramming from a transcription factor-based method to a small-molecule-driven approach, the recent progress in enhancing reprogrammed cell maturation, and the challenges associated with in vivo direct reprogramming for translational applications. It also describes our current understanding of the molecular mechanisms underlying direct reprogramming, including the role of transcription factors, epigenetic modifications, non-coding RNAs, and the function of metabolic reprogramming, and highlights novel insights gained from single-cell omics studies.

DOI: 10.1038/s41580-021-00335-z

The impact factor: 113.915

Paper 2

Title: Down-regulation of Beclin1 promotes direct cardiac reprogramming.

Abstract: Direct reprogramming of fibroblasts to alternative cell fates by forced expression of transcription factors offers a platform to explore fundamental molecular events governing cell fate identity. The discovery and study of induced cardiomyocytes (iCMs) not only provides alternative therapeutic strategies for heart disease but also sheds lights on basic biology underlying CM fate determination. The iCM field has primarily focused on early transcriptome and epigenome repatterning, whereas little is known about how reprogramming iCMs remodel, erase, and exit the initial fibroblast lineage to acquire final cell identity. Here, we show that autophagy-related 5 (Atg5)-dependent autophagy, an evolutionarily conserved self-digestion process, was induced and required for iCM reprogramming. Unexpectedly, the autophagic factor Beclin1 (Becn1) was found to suppress iCM induction in an autophagy-independent manner. Depletion of Becn1 resulted in improved iCM induction from both murine and human fibroblasts. In a mouse genetic model, Becn1 haploinsufficiency further enhanced reprogramming factor-mediated heart function recovery and scar size reduction after myocardial infarction. Mechanistically, loss of Becn1 up-regulated Lef1 and down-regulated Wnt inhibitors, leading to activation of the canonical Wnt/Î²-catenin signaling pathway. In addition, Becn1 physically interacts with other classical class III phosphatidylinositol 3-kinase (PI3K III) complex components, the knockdown of which phenocopied Becn1 depletion in cardiac reprogramming. Collectively, our study revealed an inductive role of Atg5-dependent autophagy as well as a previously unrecognized autophagy-independent inhibitory function of Becn1 in iCM reprogramming.

DOI: 10.1126/scitranslmed.aay7856

The impact factor: 19.319

Paper 3

Title: Single-cell transcriptomics for the assessment of cardiac disease.

Abstract: Cardiovascular disease is the leading cause of death globally. An advanced understanding of cardiovascular disease mechanisms is required to improve therapeutic strategies and patient risk stratification. State-of-the-art, large-scale, single-cell and single-nucleus transcriptomics facilitate the exploration of the cardiac cellular landscape at an unprecedented level, beyond its descriptive features, and can further our understanding of the mechanisms of disease and guide functional studies. In this Review, we provide an overview of the technical challenges in the experimental design of single-cell and single-nucleus transcriptomics studies, as well as a discussion of the type of inferences that can be made from the data derived from these studies. Furthermore, we describe novel findings derived from transcriptomics studies for each major cardiac cell type in both health and disease, and from development to adulthood. This Review also provides a guide to interpreting the exhaustive list of newly

identified cardiac cell types and states, and highlights the consensus and discordances in annotation, indicating an urgent need for standardization. We describe advanced applications such as integration of single-cell data with spatial transcriptomics to map genes and cells on tissue and define cellular microenvironments that regulate homeostasis and disease progression. Finally, we discuss current and future translational and clinical implications of novel transcriptomics approaches, and provide an outlook of how these technologies will change the way we diagnose and treat heart disease.

DOI: 10.1038/s41569-022-00805-7

The impact factor: 49.421

Paper 4

Title: Single-cell transcriptional profiling informs efficient reprogramming of human somatic cells to cross-presenting dendritic cells.

Abstract: Type 1 conventional dendritic cells (cDC1s) are rare immune cells critical for the induction of antigen-specific cytotoxic CD8(+) T cells, although the genetic program driving human cDC1 specification remains largely unexplored. We previously identified PU.1, IRF8, and BATF3 transcription factors as sufficient to induce cDC1 fate in mouse fibroblasts, but reprogramming of human somatic cells was limited by low efficiency. Here, we investigated single-cell transcriptional dynamics during human cDC1 reprogramming. Human induced cDC1s (hiDC1s) generated from embryonic fibroblasts gradually acquired a global cDC1 transcriptional profile and expressed antigen presentation signatures, whereas other DC subsets were not induced at the single-cell level during the reprogramming process. We extracted gene modules associated with successful reprogramming and identified inflammatory signaling and the cDC1-inducing transcription factor network as key drivers of the process. Combining IFN-Î³, IFN-Î², and TNF-Î± with constitutive expression of cDC1-inducing transcription factors led to improvement of reprogramming efficiency by 190-fold. hiDC1s engulfed dead cells, secreted inflammatory cytokines, and performed antigen cross-presentation, key cDC1 functions. This approach allowed efficient hiDC1 generation from adult fibroblasts and mesenchymal stromal cells. Mechanistically, PU.1 showed dominant and independent chromatin targeting at early phases of reprogramming, recruiting IRF8 and BATF3 to shared binding sites. The cooperative binding at open enhancers and promoters led to silencing of fibroblast genes and activation of a cDC1 program. These findings provide mechanistic insights into human cDC1 specification and reprogramming and represent a platform for generating patient-tailored cDC1s, a long-sought DC subset for vaccination strategies in cancer immunotherapy.

DOI: 10.1126/sciimmunol.abg5539

The impact factor: 30.63

Paper 5

Title: TBX20 Improves Contractility and Mitochondrial Function During Direct Human Cardiac Reprogramming.

Abstract: BACKGROUND: Direct cardiac reprogramming of fibroblasts into cardiomyocytes has emerged as a promising strategy to remuscularize injured myocardium. However, it is insufficient to generate functional induced cardiomyocytes from human fibroblasts using conventional reprogramming cocktails, and the underlying molecular mechanisms are not well studied. METHODS: To discover potential missing factors for human direct reprogramming, we performed transcriptomic comparison between human induced cardiomyocytes and functional cardiomyocytes. RESULTS: We identified TBX20 (T-box transcription factor 20) as the top cardiac gene that is unable to be activated by the MGT133 reprogramming cocktail (MEF2C, GATA4, TBX5, and miR-133). TBX20 is required for normal heart development and cardiac function in adult cardiomyocytes, yet its role in cardiac reprogramming remains undefined. We show that the addition of TBX20 to the MGT133 cocktail (MGT+TBX20) promotes cardiac reprogramming and activates genes associated with cardiac contractility, maturation, and ventricular heart. Human induced cardiomyocytes produced with MGT+TBX20 demonstrated more frequent beating, calcium oscillation, and higher energy metabolism as evidenced by increased mitochondria numbers and mitochondrial respiration. Mechanistically, comprehensive transcriptomic, chromatin occupancy, and epigenomic studies revealed that TBX20 colocalizes with MGT reprogramming factors at cardiac gene enhancers associated with heart contraction, promotes chromatin binding and co-occupancy of MGT factors at these loci, and synergizes with MGT for more robust activation of target gene transcription. CONCLUSIONS: TBX20 consolidates MGT cardiac reprogramming factors to activate cardiac enhancers to promote cardiac cell fate conversion. Human induced cardiomyocytes generated with TBX20 showed enhanced cardiac function in contractility and mitochondrial respiration.

DOI: 10.1161/CIRCULATIONAHA.122.059713

The impact factor: 39.918

Paper 6

Title: Conserved transcription factors promote cell fate stability and restrict reprogramming potential in differentiated cells.

Abstract: Defining the mechanisms safeguarding cell fate identity in differentiated cells is crucial to improve 1) - our understanding of how differentiation is maintained in healthy tissues or altered in a disease state, and 2) - our ability to use cell fate reprogramming for regenerative purposes. Here, using a genome-wide transcription factor screen followed by validation steps in a variety of reprogramming assays (cardiac, neural and iPSC in fibroblasts and endothelial cells), we identified a set of four transcription factors (ATF7IP, JUNB, SP7, and ZNF207 [AJSZ]) that robustly opposes cell fate reprogramming in both lineage and cell type independent manners. Mechanistically, our integrated multi-omics approach (ChIP, ATAC and RNA-seq) revealed that AJSZ oppose cell fate reprogramming by 1) - maintaining chromatin enriched for reprogramming TF motifs in a closed state and 2) - downregulating genes required for reprogramming. Finally, KD of AJSZ in combination with MGT overexpression, significantly reduced scar size and improved heart function by 50%, as compared to MGT alone post-myocardial infarction. Collectively, our study suggests that inhibition of barrier to reprogramming mechanisms represents a promising therapeutic avenue to improve adult organ function post-injury.

DOI: 10.1038/s41467-023-37256-8

The impact factor: 17.694

Paper 7

Title: Cross-lineage potential of Ascl1 uncovered by comparing diverse reprogramming regulatomes.

Abstract: Direct reprogramming has revolutionized the fields of stem cell biology and regenerative medicine. However, the common mechanisms governing how reprogramming cells undergo transcriptome and epigenome remodeling (i.e., regulatome remodeling) have not been investigated. Here, by characterizing early changes in the regulatome of three different types of direct reprogramming, we identify lineage-specific features as well as common regulatory transcription factors. Of particular interest, we discover that the neuronal factor Ascl1 possesses cross-lineage potential; together with Mef2c, it drives efficient cardiac reprogramming toward a mature and induced cardiomyocyte phenotype. Through ChIP-seq and RNA-seq, we find that MEF2C drives the shift in ASCL1 binding away from neuronal genes toward cardiac genes, guiding their co-operative epigenetic and transcription activities. Together, these findings demonstrate the existence of common regulators of different direct reprogramming and argue against the premise that transcription factors possess only lineage-specific capabilities for altering cell fate - the basic premise used to develop direct reprogramming approaches.

DOI: 10.1016/j.stem.2022.09.006

The impact factor: 25.269

Paper 8

Title: Cardiac regeneration by direct reprogramming in this decade and beyond.

Abstract: Japan faces an increasing incidence of heart disease, owing to a shift towards a westernized lifestyle and an aging demographic. In cases where conventional interventions are not appropriate, regenerative medicine offers a promising therapeutic option. However, the use of stem cells has limitations, and therefore, "direct cardiac reprogramming" is emerging as an alternative treatment. Myocardial regeneration transdifferentiates cardiac fibroblasts into cardiomyocytes in situ.Three cardiogenic transcription factors: Gata4, Mef2c, and Tbx5 (GMT) can induce direct reprogramming of fibroblasts into induced cardiomyocytes (iCMs), in mice. However, in humans, additional factors, such as Mesp1 and Myocd, are required. Inflammation and immune responses hinder the reprogramming process in mice, and epigenetic modifiers such as TET1 are involved in direct cardiac reprogramming in humans. The three main approaches to improving reprogramming efficiency are (1) improving direct cardiac reprogramming factors, (2) improving cell culture conditions, and (3) regulating epigenetic factors. miR-133 is a potential candidate for the first approach. For the second approach, inhibitors of TGF-Î² and Wnt signals, Akt1 overexpression, Notch signaling pathway inhibitors, such as DAPT ((S)-tert-butyl 2-((S)-2-(2-(3,5-difluorophenyl) acetamido) propanamido)-2-phenylacetate), fibroblast growth factor (FGF)-2, FGF-10, and vascular endothelial growth factor (VEGF: FFV) can influence reprogramming. Reducing the expression of Bmi1, which regulates the mono-ubiquitination of histone H2A, alters histone modification, and subsequently the reprogramming efficiency, in the third approach.

In addition, diclofenac, a non-steroidal anti-inflammatory drug, and high level of Mef2c overexpression could improve direct cardiac reprogramming.Direct cardiac reprogramming needs improvement if it is to be used in humans, and the molecular mechanisms involved remain largely elusive. Further advances in cardiac reprogramming research are needed to bring us closer to cardiac regenerative therapy.

DOI: 10.1186/s41232-021-00168-5

The impact factor: 10.426

Paper 9

Title: An Optimized Protocol for Human Direct Cardiac Reprogramming.

Abstract: Direct cardiac reprogramming, the conversion of fibroblasts into cardiomyocyte-like cells (iCMs), is an attractive approach to heal the injured heart. Here we present a new approach to human cardiac reprogramming that utilizes a polycistronic three-factor reprogramming cocktail and one microRNA. Our protocol produces cardiac Troponin T positive human iCMs (hiCMs) at an efficiency of 40%-60%, approximately double that of previous protocols, within just 2 weeks. The resulting hiCMs display cardiomyocyte-like sarcomere structure, gene expression, and calcium oscillation. For complete details on the use and execution of this protocol, please refer to Zhou et al. (2019).

DOI: 10.1016/j.xpro.2019.100010

The impact factor: 0.0

Paper 10

Title: Optimized protocol for direct cardiac reprogramming in mice using Ascl1 and Mef2c.

Abstract: Direct cardiac reprogramming refers to the conversion of fibroblasts into cardiomyocyte-like cells (iCMs) without going through an intermediate progenitor stage. Here, we present a protocol for direct cardiac reprogramming in mice using Ascl1 and Mef2c. We describe steps for isolating primary neonatal mouse cardiac fibroblast, preparing retrovirus encoding reprogramming factors, and efficient cardiac reprogramming with Ascl1 and Mef2c. The resulting iCMs display cardiomyocyte-like sarcomere structure, gene expression, and calcium flux. For complete details on the use and execution of this protocol, please refer to Wang etÂ al. (2022).(1).

DOI: 10.1016/j.xpro.2023.102204

The impact factor: 0.0