Report Title

Introduction

Introduction:

The research on single-cell transcriptomic analysis of human embryonic development is of immense significance in unraveling the molecular mechanisms of cell fate determination, particularly in the context of cardiac reprogramming. This research aims to understand the regulatory networks involved in cell fate determination during cellular reprogramming, with a specific focus on the interplay between Becn1 and Wnt signaling pathways.

The importance of this research stems from the need to comprehend the intricate mechanisms governing cell fate decisions, shedding light on previously uncharacterized roles of Becn1 and Wnt signaling pathways in cellular reprogramming. Understanding these roles provides mechanistic insights into autophagy-dependent and -independent regulatory networks, with potential implications for regenerative medicine strategies targeting cell fate determination.

The scope of the research encompasses the identification of Becn1-binding partners and their involvement in biological processes related to pathways regulating cell adhesion, cytoskeleton organization, nucleation, endocytosis, and macroautophagy. The study also delves into the dysregulation of Becn1 in various cardiovascular diseases, emphasizing the clinical relevance and necessity of understanding its role in cell fate determination.

The potential impact of this study extends to developmental biology, regenerative medicine, and molecular biology, offering new perspectives on cellular reprogramming and therapeutic strategies for cardiac regeneration. Additionally, the research findings may contribute to the development of novel therapeutic strategies and interventions targeting cell fate determination in various biological contexts.

The significance of this research is underscored by the unexpected findings that challenge the conventional understanding of Becn1's role and highlight the complexity of regulatory networks governing cell fate determination. The potential implications of this research for regenerative medicine, developmental biology, and the broader understanding of cellular reprogramming underscore the critical importance of this research area.

Literature

depletion on iCM generation and functionality, providing insights into the impact of Becn1 on in vivo reprogramming efficiency. The results suggest that Becn1 plays a critical role in inhibiting iCM reprogramming, and its depletion enhances the efficiency of iCM conversion.

In conclusion, the research provides a comprehensive understanding of the molecular mechanisms underlying cell fate determination during human embryonic development, shedding light on the role of Becn1 and autophagy in iCM reprogramming. The findings contribute to the existing literature on cell fate determination and have implications for the understanding of regulatory mechanisms involved in human embryonic development.

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/

Discussion

oblast to cardiomyocyte. Furthermore, the study identified the ULK1–PI3K complex–Wnt/β-catenin signaling network as a previously unrecognized regulatory mechanism, paving the way for a deeper understanding of the molecular pathways governing cell fate determination.

Comparisons with other studies in related fields revealed the broader implications of the findings, particularly in the context of cardiovascular diseases. The dysregulation of Becn1 has been associated with various cardiovascular conditions, highlighting the relevance of the research findings in the broader context of cardiac function regulation.

However, the study has limitations, including the need for further exploration of the multifaceted roles of Becn1 in cellular stress, apoptosis, oxidative stress, and epithelial-mesenchymal transition for successful cell fate determination and maintenance. Future research directions could involve investigating the broader functions of MGT-induced autophagy, further elucidating the molecular mechanisms underlying Becn1 regulation of cardiac function, and exploring the specific interactions between Becn1, ULK1, PI3K complex, and Wnt/β-catenin signaling network.

In summary, the study significantly contributes to the understanding of cell fate determination mechanisms and provides valuable insights into the roles of Becn1 and the associated regulatory pathways in induced cardiomyocyte fate acquisition. The findings have implications for cardiovascular health and pave the way for future research endeavors aimed at unraveling the complexities of cell fate determination in human embryonic development.

In conclusion, the research provides novel insights into the molecular mechanisms underlying cell fate determination, particularly in the context of induced cardiomyocyte fate acquisition. The study's findings contribute to the understanding of regulatory networks involved in cell fate acquisition and provide a basis for future research targeting the manipulation of cell fate for therapeutic purposes in regenerative medicine.

Idea

Based on the provided target paper, "Single-Cell Transcriptomic Analyses of Cell Fate Transitions during Human Cardiac Reprogramming," and its related papers, a potential research problem could be:

Problem: Understanding the Role of Autophagy-Related Mechanisms in Enhancing Human Cardiac Reprogramming and Identifying Potential Therapeutic Targets.

Rationale: The target paper highlights the importance of immune-response-associated DNA methylation in human induced cardiac myocyte (hiCM) induction, while one of the related papers emphasizes the significant role of autophagy-related 5 (Atg5)-dependent autophagy and autophagy-independent functions of Beclin1 (Becn1) in inducing cardiac reprogramming. Understanding the molecular mechanisms underlying autophagy-related pathways in enhancing hiCM induction and how they intersect with immune-response-associated DNA methylation could provide novel insights into improving the efficiency and efficacy of cardiac reprogramming. Identifying potential therapeutic targets within these pathways holds promise for advancing regenerative medicine strategies for heart disease.

Method

To address the specified research problem, a scientific method can be developed as follows:

1. Review and integrate existing findings: A systematic review of the target paper and its related studies should be conducted. This will provide a thorough understanding of the molecular mechanisms underlying hiCM (human induced cardiac myocyte) determination, the role of immune-response-associated DNA methylation, autophagy-related mechanisms involving Atg5-dependent autophagy and autophagy-independent functions of Beclin1 (Becn1) in inducing cardiac reprogramming.

2. Hypothesis Formation: Following the review of existing findings, a hypothesis should be formed based on the potential interaction between Atg5-dependent autophagy and autophagy-independent functions of Becn1, and immune-response-associated DNA methylation, with a specific focus on how these mechanisms enhance human cardiac reprogramming.

3. Design a Comprehensive Experimental Plan: Develop a structured and rigorous experimental plan to address the research problem and test the formulated hypothesis. Considering the complexity of the problem, integrating different approaches (molecular biology, biochemistry, single-cell transcriptomics, and functional screening) will be necessary to elucidate the interactions and potential therapeutic targets within autophagy-related pathways.

4. Method Development: This is an essential part of the scientific method, encompassing the design and implementation of experiments aligned with the hypothesis. The development should include specific assays or techniques to target the interactions between Atg5-dependent autophagy, autophagy-independent functions of Becn1, and immune-response-associated DNA methylation.

5. Conduct Valid and Reproducible Experiments: Rigorous experiments should be conducted to collect reliable and valid data, ensuring that the potential outcomes are directly aligned with the hypothesized interaction and the research problem. Experimental designs should include control groups and replicates to validate the findings.

6. Data Analysis and Interpretation: A statistically robust method should be developed to analyze the experimental data, considering the complexity of the interactions and their respective effects on human cardiac reprogramming efficiency. This analysis should allow for a comprehensive interpretation of the findings and address the research problem.

By following the aforementioned method, the interactions between Atg5-dependent autophagy, autophagy-independent functions of Becn1, and immune-response-associated DNA methylation in enhancing human cardiac reprogramming can be systematically and rigorously explored. The findings will not only address the research problem but also contribute to advancing regenerative medicine strategies for heart disease, with potential implications for the broader field of cell fate determination and reprogramming.

Experiment

Experiment:

Title:

Exploring the Interactions of Autophagy-Related Mechanisms in Enhancing Human Cardiac Reprogramming and Identifying Potential Therapeutic Targets

Objective:

To systematically investigate the role of autophagy-related mechanisms, including Atg5-dependent autophagy and autophagy-independent functions of Beclin1 (Becn1), in enhancing human cardiac reprogramming and to identify potential therapeutic targets within these pathways.

Rationale:

The experiment aims to address the research problem by elucidating the molecular mechanisms underlying the interactions between autophagy-related pathways and immune-response-associated DNA methylation in enhancing human cardiac reprogramming. This understanding is crucial for advancing regenerative medicine strategies for heart disease and can lead to the identification of novel therapeutic targets.

Experimental Design:

1. Systematic Review of Existing Findings:

Conduct a thorough review of the target paper and its related studies to gain a comprehensive understanding of the molecular mechanisms underlying hiCM determination, the role of immune-response-associated DNA methylation, and the involvement of autophagy-related mechanisms in inducing cardiac reprogramming.

2. Hypothesis Formation:

Based on the reviewed findings, formulate a hypothesis regarding the potential interaction between Atg5-dependent autophagy, autophagy-independent functions of Becn1, and immune-response-associated DNA methylation, focusing on their collective role in enhancing human cardiac reprogramming.

3. Experimental Plan Development:

Develop a structured experimental plan integrating molecular biology, biochemistry, single-cell transcriptomics, and functional screening approaches to systematically elucidate the interactions and potential therapeutic targets within autophagy-related pathways.

4. Method Development:

Design specific assays and techniques to target the interactions between Atg5-dependent autophagy, autophagy-independent functions of Becn1, and immune-response-associated DNA methylation, allowing for the detailed exploration of their effects on human cardiac reprogramming efficiency.

5. Conduct of Valid and Reproducible Experiments:

Rigorously conduct experiments to collect reliable and valid data, ensuring that the outcomes are aligned with the hypothesized interaction and the research problem. Include appropriate control groups and replicates to validate the findings.

6. Data Analysis and Interpretation:

Develop a statistically robust method to analyze the experimental data, accounting for the complexity of the interactions, and their respective effects on human cardiac reprogramming efficiency. This analysis should allow for a comprehensive interpretation of the findings and address the research problem effectively.

Expected Outcome:

The experiment is expected to provide novel insights into the role of autophagy-related mechanisms in enhancing human cardiac reprogramming, potentially leading to the identification of specific targets for therapeutic intervention in regenerative medicine strategies for heart disease.

By following this experimental design, the interactions between Atg5-dependent autophagy, autophagy-independent functions of Becn1, and immune-response-associated DNA methylation can be systematically and rigorously explored, contributing significantly to the advancement of the field.

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