In Vitro Study of Notch1 Signaling in Cardiomyocyte Differentiation, Maturation, and Gene Expression Regulation

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Abstract: This study investigates the role of Notch1 signaling in cardiomyocyte differentiation, maturation, and gene expression regulation. Using neonatal mice with floxed Notch1 genes, Cre recombinase expression was controlled by MLC2v to achieve Notch1 knockout, supplemented by γ-secretase inhibitor DAPT and gene transfection to construct various cardiomyocyte models. Techniques such as immunofluorescence staining, Western blot, Myomesin staining, and quantitative RT-PCR were used to analyze Notch1 signaling activation and changes in differentiation-related genes Jagged1 and Hes1. Results revealed that inhibiting Notch1 signaling significantly promoted cardiomyocyte differentiation and maturation, upregulating cardiac differentiation markers Jagged1 and Hes1. Conversely, Notch1 overexpression enhanced signaling, inhibiting differentiation and downregulating related genes. Mechanical stretch enhanced Notch1 activation and inhibited differentiation, while NSAID intervention had opposite effects. These findings demonstrate the crucial role of Notch1 signaling in cardiomyocyte differentiation, with implications for cardiac development and disease research.

Keywords: Notch1 Signaling; Cardiomyocytes; Differentiation and Maturation; Gene Expression; In Vitro Study

1. Introduction

1.1 Overview of the Notch1 Signaling Pathway

The Notch signaling pathway is a crucial mode of intercellular communication, playing a significant role in regulating various biological processes. Notch1, in particular, functions as a regulator of cell differentiation, proliferation, and apoptosis. As a transmembrane receptor, Notch1 undergoes ligand-dependent cleavage, releasing the activated Notch1 intracellular domain (NICD) that translocates to the nucleus to regulate transcription. Activation and termination of this signaling are mediated by γ-secretase and the regulation of Notch1 gene expression, essential for maintaining tissue homeostasis and promoting specific cell differentiation.

1.2 Importance of Cardiomyocyte Differentiation and Maturation

Cardiomyocyte differentiation and maturation are pivotal in cardiac development and a key focus in regenerative medicine. This process involves the synthesis of structural proteins, formation of functional channels, and precise regulation of signaling pathways. Immature cardiomyocytes lack sufficient contractile capacity and electrophysiological function, impairing cardiac operation. Understanding the molecular mechanisms of cardiomyocyte differentiation and maturation can aid in developing new cardiac regenerative therapies. the maturity of cardiomyocytes is also closely linked to cardiac disease development, making the study of Notch1 signaling's impact in vitro essential.

1.3 Review of Current Research

Notch1's role in cardiomyocyte development has garnered significant attention globally. International studies indicate that Notch1 not only inhibits premature cardiomyocyte differentiation but also contributes to the pathology of cardiac malformations and cardiomyopathy. Domestic research has begun to uncover the complex regulatory mechanisms of the Notch1 pathway and its applications in cardiac regenerative medicine. However, controversies remain, especially concerning Notch1's interactions with other pathways.

Thus, in-depth studies using in vitro models to explore Notch1's role in cardiomyocyte differentiation are theoretically and practically significant.

2. Materials and Methods

2.1 Experimental Materials

The study utilized neonatal mice with floxed Notch1 genes, where MLC2v-controlled Cre recombinase specifically knocked out the Notch1 gene in cardiomyocytes. Differentiation and non-differentiation media were used as needed. Antibodies against Notch1 (n1ic) and myomesin were employed to detect Notch1 signaling and cardiomyocyte differentiation. γ-secretase inhibitor DAPT and NSAIDs were used to regulate Notch1 activity. RNA extraction kits, reverse transcription reagents, and quantitative PCR kits were used for gene expression analysis.

2.2 Cardiomyocyte Isolation and Culture

Cardiomyocytes were isolated through enzymatic digestion of neonatal mouse heart tissue. the isolated cells were cultured in differentiation and non-differentiation media to observe Notch1's effects on different differentiation states under controlled conditions to ensure cell viability and experimental reproducibility.

2.3 Model Establishment

2.3.1 Floxed Notch1 Gene Mouse Model

Using MLC2v-controlled Cre recombinase, Notch1 gene knockout was achieved in cardiomyocytes, facilitating the study of Notch1 deficiency on cardiomyocyte differentiation and maturation.

2.3.2 DAPT Treatment Model

Γ-secretase inhibitor DAPT was used to block Notch1 signaling in cultured cardiomyocytes, simulating chemical inhibition of Notch1 to understand its role in differentiation.

2.3.3 Notch1 Gene Overexpression Model

Gene transfection techniques were used to overexpress Notch1 in cardiomyocytes to assess the effects of enhanced Notch1 signaling on cell differentiation and maturation.

2.4 Notch1 Signaling Detection

2.4.1 Immunofluorescence Staining

Immunofluorescence staining using Notch1(n1ic) antibodies qualitatively assessed Notch1 activation, visually displaying changes under different experimental conditions.

2.4.2 Western Blot Analysis

Western blotting quantitatively measured Notch1 protein expression, providing precise data on intracellular Notch1 expression changes.

2.5 Cardiomyocyte Differentiation Detection

2.5.1 Myomesin Staining

Myomesin antibodies were used for immunostaining to evaluate cardiomyocyte differentiation and maturation, with expression levels correlating to cellular maturity.

2.5.2 Quantitative RT-PCR

RNA was extracted and reverse-transcribed to cDNA for quantitative RT-PCR analysis of cardiac differentiation markers Jagged1 and Hes1, using relative quantification to display expression trends.

2.6 Mechanical Stretch and NSAID Intervention Experiments

Mechanical stretch was applied using a cell stretcher to simulate cardiac mechanical stress, assessing Notch1's response and its impact on cardiomyocyte differentiation. NSAIDs provided a method to investigate inflammatory signaling intervention, significantly affecting Notch1 activity and promoting cardiomyocyte maturation.

3. Results

3.1 Role of Notch1 Signaling in Cardiomyocyte Differentiation

3.1.1 Floxed Notch1 Gene Mouse Model Results

Notch1 knockout via Cre recombinase resulted in significantly decreased Notch1 activity, as shown by reduced immunofluorescence signals. Myomesin staining indicated enhanced cardiomyocyte differentiation and maturation. Quantitative RT-PCR showed a 2-3 fold increase in Jagged1 and Hes1 expression ($P<0.05$), highlighting Notch1 inhibition as a key factor in promoting maturation.

3.1.2 DAPT Treatment Model Results

DAPT treatment significantly lowered Notch1 activity, enhancing differentiation as shown by increased myomesin staining and upregulated Jagged1 and Hes1 expression ($P<0.05$), consistent with floxed Notch1 model results.

3.1.3 Notch1 Gene Overexpression Model Results

Overexpression enhanced Notch1 signaling, reducing differentiation and maturation, evidenced by decreased myomesin staining and Jagged1 and Hes1 expression ($P<0.05$), indicating Notch1's inhibitory role in maturation.

3.2 Effects of Mechanical Stretch and NSAID on Differentiation and Notch1 Signaling

3.2.1 Mechanical Stretch Results

Mechanical stress increased Notch1 activity, inhibiting differentiation as shown by reduced myomesin staining and lower Jagged1 and Hes1 expression ($P<0.05$), highlighting mechanical stress's potential impact under physiological and pathological conditions.

3.2.2 NSAID Intervention Results

NSAIDs reduced Notch1 activity, enhancing differentiation with increased myomesin staining and Jagged1 and Hes1 expression ($P<0.05$), suggesting a therapeutic potential in cardiac disease treatment by modulating Notch1 signaling.

4. Discussion

4.1 Mechanisms of Notch1 Signaling in Cardiomyocyte Differentiation and Maturation

The Notch1 signaling pathway plays a multifaceted role in biological development, particularly impacting cardiomyocyte differentiation and maturation. Our studies using Floxed Notch1 gene mouse models, DAPT treatment, and Notch1 overexpression models reveal the negative regulatory nature of Notch1 in this process. Consistent with its role in other tissues, Notch1 signaling maintains cells in an undifferentiated state by selectively inhibiting or activating specific genomic targets. One potential mechanism is the suppression of cardiomyocyte-specific transcription factors, hindering their progression to mature cardiomyocytes. Additionally, Notch1 might interact with pathways like Wnt and BMP, influencing cardiomyocyte developmental pathways. These interactions likely involve complex feedback regulation that warrants further mechanistic investigation to elucidate its biological effects and regulatory networks.

4.2 Mechanisms and Significance of Mechanical Stretch and NSAID Intervention

The dynamic changes in the physical environment of cardiomyocytes significantly influence cardiac development and disease progression. Mechanical stretch in our experiments enhanced Notch1 signaling, indicating that physical stress can activate the pathway via mechanotransduction mechanisms. This might occur through membrane changes that promote Notch1 receptor-ligand interactions, potentially involving cytoskeletal remodeling and mechanical stress-related proteins. Moreover, NSAID's inhibitory effect on Notch1 suggests that inflammatory signals may regulate cardiomyocyte maturation via Notch1 modulation. By inhibiting pro-inflammatory mediators such as prostaglandins, NSAIDs might reduce Notch1 activity, promoting differentiation. Given NSAIDs' widespread clinical use, understanding their role in cardiac cell differentiation has scientific significance and potential implications for new cardiovascular disease treatments.

4.3 Limitations and Future Research Directions

While this study advances our understanding of Notch1's regulatory role in cardiomyocyte differentiation, limitations exist. the in vitro conditions differ from complex in vivo environments, potentially affecting pathway interactions and physiological effects. the regulation of Notch1 signaling is influenced by various internal and external factors, and single-factor studies may not fully capture its function in physiological and pathological states. Future research should validate in vitro findings with in vivo studies and employ genomics and proteomics to explore the dynamic regulation of Notch1 in cardiomyocyte differentiation. Investigating interactions with other signaling pathways will aid in comprehensively understanding developmental network mechanisms, providing insights for cardiovascular diagnostics and treatment strategies.

5. Conclusion

This study demonstrates the negative regulatory role of Notch1 signaling in cardiomyocyte differentiation and maturation through various models. Changes in Notch1 activity significantly affect cardiomyocyte maturity, with mechanical stretch and NSAID interventions offering new perspectives on

differentiation under different conditions. While limitations exist, our findings provide experimental evidence for Notch1's role in cardiomyocyte differentiation. Future research should explore Notch1's interactions with other pathways and its dynamic regulation in vivo, laying the groundwork for cardiac regenerative medicine and cardiovascular treatment development. Notch1 signaling holds potential as a regulatory target in cardiology, providing new approaches and methods for managing related diseases.

Funding Information
Supported by Inner Mongolia Natural Science Foundation (2020MS08139); Inner Mongolia Health Commission Medical and Health Technology Project (202201195).

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