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Role and Mechanism of Notch1 in Cardiomyocyte Response to Mechanical Stretch in Hypertensive Cardiac Hypertrophy and NSAID Intervention

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Abstract: This study investigates the role and mechanisms of Notch1 in cardiomyocytes under mechanical stretch in the context of hypertensive cardiac hypertrophy and NSAID intervention. Using adult Sprague-Dawley rats, cardiomyocytes were isolated and cultured to establish a mechanical stretch model. Various intensities of mechanical stretch were applied to observe the expression and distribution of Notch1 and related signaling proteins (e. g., Hes1, KLF2, FABP4). Techniques such as Western blot, ELISA, immunofluorescence, electron microscopy, and RT-PCR were employed to comprehensively assess cardiomyocyte responses. Experimental groups included controls, mechanical stretch, siRNA, 50 μM DAPT, and Notch1 overexpression, with data collected at multiple time points. Results indicated that mechanical stretch significantly activated the Notch signaling pathway, promoting cardiomyocyte hypertrophy. In contrast, siRNA-mediated Notch1 silencing, DAPT inhibition of the Notch pathway, or NSAID treatment effectively suppressed stretch-induced hypertrophy. Notch1 plays a crucial role in cell-cell interactions, alignment, and proliferation, possibly regulating cardiomyocyte structure and function. NSAIDs, by modulating the Notch pathway, markedly inhibit cardiac hypertrophy, suggesting a novel therapeutic approach for hypertensive cardiac hypertrophy.

Keywords: Notch1; Mechanical Stretch; Cardiac Hypertrophy; NSAID; Signaling Pathway

1. Introduction

1.1 Research Background and Significance

Cardiac hypertrophy is a key indicator of structural and functional changes in the heart due to hypertension. Elevated blood pressure imposes persistent mechanical load on cardiomyocytes, leading to hypertrophy and fibrosis, which are closely associated with severe cardiovascular events like heart failure and arrhythmias. Understanding the mechanisms of hypertrophy and finding effective interventions are crucial in cardiology. the Notch1 signaling pathway, a highly conserved mechanism, plays a critical role in cell differentiation, proliferation, and apoptosis. Abnormal activation of Notch1 in cardiomyocytes may contribute to hypertrophy. Furthermore, the role of NSAIDs in hypertrophy remains unclear, warranting exploration of Notch1's role under mechanical stress and NSAID intervention.

1.2 Overview of the Notch1 Signaling Pathway

The Notch signaling pathway is vital in various biological processes, including cardiac development and pathology. Activation occurs when the Notch1 receptor interacts with its ligands Jagged or Delta, releasing the Notch intracellular domain, which enters the nucleus to regulate gene transcription. Abnormal Notch signaling is linked to cardiovascular diseases such as hypertrophy and fibrosis. Detailed molecular mechanisms of Notch1 in cardiac remodeling require further study to potentially provide new therapeutic targets.

1.3 Effects of Mechanical Stretch on Cardiomyocytes

Mechanical stretch is a common stress experienced by cardiomyocytes under

physiological and pathological conditions. It influences proliferation and function through pathways such as MAPK, PI3K/Akt, and Notch1. Increased stretch stress leads to cytoskeletal remodeling and hypertrophy. the signaling activated by mechanical stretch may be associated with changes in extracellular matrix proteins and adhesion molecules, promoting hypertrophy.

1.4 Potential Role of NSAIDs in Cardiac Hypertrophy

NSAIDs, through COX inhibition, reduce prostaglandin production, thus exhibiting anti-inflammatory and analgesic effects. Recent studies suggest complex cardiovascular roles, with unclear impacts on hypertrophy. NSAIDs might influence cardiac remodeling by modulating inflammation and signaling pathways. Investigating NSAID effects on the Notch1 pathway could reveal therapeutic potential in hypertrophy.

1.5 Research Objectives and Questions

This study aims to elucidate the role of the Notch1 pathway in hypertrophy under mechanical stress and explore NSAID effects. Key questions include: (1) Mechanisms of Notch1 pathway activation by mechanical stretch, (2) Pathways through which NSAIDs affect Notch1 and hypertrophy, (3) Regulation of cell junctions, alignment, and proliferation by Notch1 and NSAIDs during hypertrophy.

2. Materials and Methods

2.1 Experimental Materials

Standardized materials ensure reproducibility and data reliability, including collagenase II, trypsin, DMEM, and fetal bovine serum for cardiomyocyte isolation and culture. Western blot and ELISA kits assess protein expression and inflammatory factors. siRNA and Notch1 plasmids purchased from Sigma-Aldrich facilitate genetic interventions.

2.2 Animal Selection and Handling

Healthy adult Sprague-Dawley rats (250-300g), irrespective of sex, were used. They were housed in controlled environments, following ethical guidelines to ensure welfare.

2.3 Cardiomyocyte Isolation and Culture

Rat cardiomyocytes were isolated using collagenase II and trypsin, then cultured in DMEM with 10% fetal bovine serum. Cultures were maintained in incubators regulating temperature and CO2.

2.4 Mechanical Stretch Model Establishment

Mechanical stretch models were created using silicone dish and centrifugation methods to simulate physiological and pathological stress environments on cardiomyocytes.

2.5 Experimental Group Design

Groups included controls, mechanical stretch, siRNA, DAPT, and Notch1 overexpression. Samples were collected at different times (24h, 48h, 72h) for thorough analysis.

2.6 Protein Expression and Pathway Detection

Western blotting assessed Notch1 and related proteins. Proteins extracted post-lysis were subjected to SDS-PAGE and transferred to PVDF membranes for specific antibody detection and ECL visualization.

2.7 Inflammatory Factor Level Detection

ELISA kits measured levels of TNF-α, IL-10, and IL-6 in culture supernatants, with triplicate experiments ensuring reliability.

2.8 Cell Morphology and Ultrastructure Observation

Fluorescence microscopy and electron microscopy examined cell morphology and structure. Immunofluorescence assessed protein localization, while electron microscopy provided ultrastructural insights.

2.9 Data Analysis Methods

SPSS software was used for statistical analysis, selecting appropriate tests like t-test or ANOVA. Data were reported as mean ± SD, with $P<0.05$ indicating significance.

3. Experimental Results

3.1 Effects of Mechanical Stretch on Notch1 Signaling Pathway

Mechanical stretch significantly upregulated Notch1 expression in cardiomyocytes. Western blot analysis showed a 2.5-fold increase in Notch1 protein levels compared to controls ($P<0.01$), with a 3-fold rise in downstream

target Hes1 (P<0.01). Immunofluorescence revealed enhanced nuclear localization of the Notch intracellular domain (NICD), consistent with increased transcriptional activity. Electron microscopy indicated enlarged nucleoli, suggesting heightened transcriptional activity aligned with Notch1 pathway activation.

3.2 Inhibition of Cardiomyocyte Hypertrophy by siRNA and DAPT

siRNA-mediated silencing reduced Notch1 protein levels by over 70% (P<0.001) and significantly downregulated Hes1 and KLF2 expression. DAPT treatment decreased key protein expression in the Notch1 pathway by 50% (P<0.01) and reduced cell size by approximately 30% (P<0.05), mitigating hypertrophy. RT-PCR confirmed significant mRNA level changes, supporting Notch1's critical role in hypertrophy.

3.3 NSAID Intervention on Cardiomyocyte Hypertrophy

NSAID treatment decreased Notch1 protein expression by about 40% (P<0.01), reduced TNF-α and IL-6 levels by 35% and 50% respectively (P<0.01), and increased IL-10 by 20% (P<0.05). These results underscore NSAID's anti-inflammatory effects and potential mechanism in hypertrophy intervention. Fluorescence microscopy showed improved cell alignment and tighter cell junctions post-NSAID treatment, indicating structural benefits.

3.4 Role of Notch1 in Cell Junctions and Alignment

Notch1 activation was linked to changes in cell junctions and alignment. Mechanical stretch increased cell spacing by 50% (P<0.01) and disrupted adhesion protein expression, indicating cytoskeletal reorganization. Notch1 overexpression led to irregular cell alignment and increased spacing, observed by immunofluorescence, suggesting Notch1's role in cytoskeletal and adhesion protein regulation.

4. Discussion

4.1 Mechanism of Notch1 Activation by Mechanical Stretch

Mechanical stretch, a significant biomechanical stress, activates Notch1 by altering receptor conformation upon ligand binding, promoting NICD release and gene activation. This regulation is vital for cellular adaptation under stress. Cytoskeletal reorganization enhances signal efficiency, sustaining Notch1 activation for adaptation.

4.2 Mechanisms of siRNA, DAPT, and NSAID Effects on Hypertrophy

siRNA reduces Notch1 mRNA, decreasing protein synthesis, while DAPT inhibits γ-secretase, blocking NICD release. NSAIDs, through COX inhibition, reduce prostaglandin and inflammation, indirectly affecting Notch1 signaling. ELISA showed decreased TNF-α and IL-6 and increased IL-10 post-NSAID treatment, supporting its protective mechanism.

4.3 Notch1's Role in Structural and Functional Regulation

Notch1 influences cytoskeletal reorganization and adhesion protein expression, affecting cell morphology and alignment. Notch1 activation disrupted cell junction stability and increased nuclear transcriptional activity, impacting hypertrophy and differentiation processes.

4.4 Clinical Implications and Limitations

Findings highlight Notch1's role in hypertrophy and potential of siRNA and DAPT as interventions. NSAIDs may offer cardiovascular benefits, though clinical translation requires careful consideration due to possible side effects and physiological differences. Further trials are needed for validation.

5. Conclusion

The study elucidates mechanical stretch's role in activating Notch1 signaling in hypertrophy, demonstrating Notch1's regulatory function in cellular enlargement and remodeling. siRNA and DAPT effectively mitigate hypertrophy, while NSAIDs exhibit potential therapeutic effects through anti-inflammatory mechanisms, offering new insights for hypertrophy treatment strategies.

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