CHRD 2024: Abstract Submission Form

Presenter Name
Gauri Bhardwaj

Presenter Status
Undergraduate Students

Role in the project
Design
Perform Experiments
Analyze Data
Write Abstract

Research Category
Basic Science

Title
Control of cardiac fibroblast differentiation by Zeb1 and Zeb2

Background
Mowat Wilson Syndrome (MOWS) is a developmental disorder characterized by intellectual disability and heart defects. Mutations in the transcription factor ZEB2 causes MOWS. ZEB2 regulates the migration, proliferation, and contractility of cardiac fibroblasts.

Objective
Our study's aim was to determine if there is a negative feedback loop in fibroblasts between ZEB2 and a related gene ZEB1, in which decreased expression of one gene result in the increased expression of the other.

Methods
In NIH 3T3 embryonic fibroblasts, siRNA mediated knockdown of ZEB1 and ZEB2 was performed. Cells were transfected with 4 different siRNAs targeting ZEB1 or ZEB2 using Lipofectamine. Transfected cells were harvested 48 hours later, protein concentrations were measured, and samples were analyzed by Western Blotting. The expressions of ZEB1, ZEB2, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and α-smooth muscle actin (α-SMA), were compared in ZEB1 and ZEB2 knockdown samples.

Results
Analysis of Western Blotting showed that ZEB1 knockdown was successful, with a knockdown of 80% achieved. Contrasting previous results, ZEB2 expression in ZEB1 knockdown samples remained unchanged. Similarly, α-SMA expression did not significantly change upon ZEB1 knockdown. ZEB2 samples were not effectively knocked down by the 4 siRNAs tested. A positive control siRNA transfection successfully reduced GAPDH expression, showing the transfection procedure was effective.

Conclusion
From our results, we conclude that loss of ZEB1 does not result in a compensatory increase in ZEB2 levels in NIH 3T3 mouse embryonic fibroblasts. No change was observed in expression of ZEB2 upon ZEB1 knock down. The lack of ZEB2 knockdown prevented a determination whether expression of ZEB1 or α-SMA expression was dependent on ZEB2. We plan on repeating ZEB2 knockdown with new siRNAs and completing double knockdown studies to examine if ZEB1 and ZEB2 compensate for each other. Our findings emphasize the need for further testing on how ZEB1/2 autoregulation may affect cellular processes, that contribute to developmental diseases like MOWS.

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No

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