Investigating the Effect of microRNA-21 Deletion on Western Diet–Induced SMAD Signaling and Fibrotic Marker Expression in Mice

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ABSTRACT

Introduction: Cardiovascular disease (CVD) is characterized by endothelial dysfunction and vascular fibrosis, with diet serving as a major modifiable risk factor. MicroRNA-21 (miR-21) has been implicated in vascular remodeling and fibrotic signaling, but its role in diet-induced endothelial dysfunction remains unclear. Here, we investigated whether miR-21 deletion alters SMAD-dependent signaling and collagen deposition in mice exposed to a Western (high-fat) diet.

Methods: Wild-type (WT) and global miR-21 knockout (miR-21$^{-/-}$) mice were fed either a control diet (CD) or Western diet (WD) for 18 weeks. Protein expression of total and phosphorylated SMAD2 and SMAD3, as well as collagen type I (Col1) and type III (Col3), was measured by Western blot and normalized to GAPDH (n = 12 mice per group). Statistical significance was determined for diet and genotype comparisons.

Results: WD exposure increased SMAD2 phosphorylation in WT mice, indicating enhanced profibrotic signaling. In contrast, miR-21$^{-/-}$ mice displayed attenuated SMAD2 and altered SMAD3 phosphorylation under both diets. Col1 expression was elevated in miR-21$^{-/-}$ mice regardless of diet, whereas Col3 was increased only in WT mice fed WD. These findings suggest that miR-21 promotes diet-induced SMAD activation and selectively modulates collagen deposition, potentially contributing to vascular fibrosis.

Conclusions: Our data indicate that miR-21 is a potential mediator of diet-induced vascular fibrotic signaling through SMAD-dependent pathways. Targeting miR-21 may provide a therapeutic strategy to mitigate early molecular events leading to fibrosis and CVD progression.

Hypothesis: MiR-21$^{-/-}$ mice will exhibit blunted SMAD2/3 phosphorylation, leading to altered collagen I/III deposition and attenuation of molecular pathways associated with vascular fibrosis.

INTRODUCTION

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality and is often associated with vascular fibrosis and extracellular matrix remodeling. Activation of transforming growth factor-β (TGF-β) signaling promotes phosphorylation of SMAD2 and SMAD3, which regulate transcription of fibrotic genes including collagen types I and III (Chen et al., 2025). MicroRNA-21 (miR-21) is a small non-coding RNA that regulates gene expression at the post-transcriptional level and has been implicated in cardiovascular remodeling and fibrotic signaling pathways (Cheng & Zhang, 2010). However, the role of miR-21 in diet-induced activation of SMAD-dependent fibrosis remains unclear. Therefore, we investigated whether deletion of miR-21 alters SMAD signaling and collagen expression in mice exposed to a Western diet.

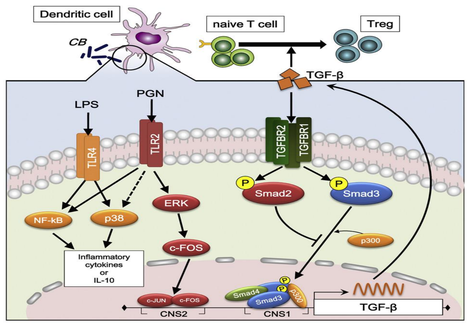

Figure 1. Overview of SMAD-dependent signaling. Ligand binding to transforming growth factor-beta (TGF-β) receptors induces phosphorylation of receptor-regulated SMADs (SMAD2 and SMAD3), which then form a complex with SMAD4 and translocate to the nucleus to regulate transcription of profibrotic genes, including collagen types I and III. This pathway contributes to extracellular matrix remodeling and vascular fibrosis. (Kashiwagi et al., 2015)

METHODS

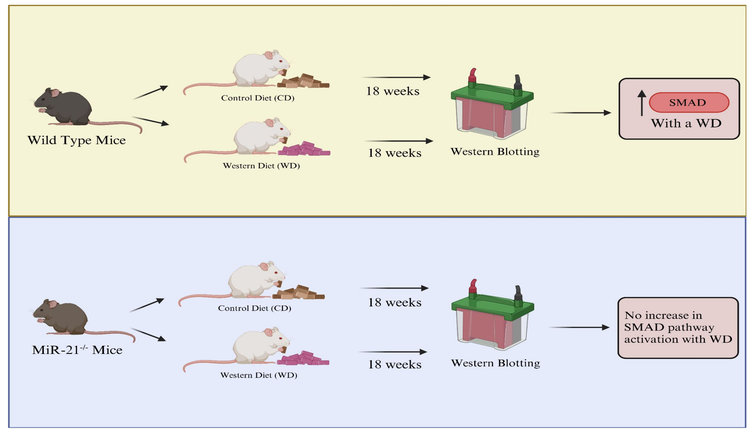

Figure 2. Overview of Mice Intervention.

- 48 male mice at an age of 8 weeks were split into two diet groups for both wild type and miR-21$^{-/-}$.
- The control diet groups were fed a standard mice chow diet (3.67 kcal/g, 12.7% kcal fat, 72.4% kcal carbohydrate, 150 g/kg sucrose).
- The western diet group was fed high-fat, high-sucrose diet (4.68 kcal/g, 44.6% kcal fat, 40.7% kcal carbohydrate, 340 g/kg sucrose).
- Dietary interventions lasted 18 weeks. After 18 weeks, experimental protocols were conducted.
- Mice were first anesthetized with ketamine injections and penile tissue was harvested.
- During extraction, the corpus spongiosum, dorsal vein, and connective tissue was removed.
- The corpus cavernosum was collected and was snap-frozen in liquid nitrogen and stored at -80℃.
- The corpus cavernosum was homogenized and 20 µg of protein were loaded into each lane for western blotting.

The following proteins and their respective dilutions were used:

- Col 1 - 1:5000
- Col 3 - 1:1000
- P-SMAD2 - 1:2000
- SMAD2 - 1:2000
- P-SMAD3- 1:1000
- SMAD3 - 1:2000
- GAPDH - 1:4000

RESULTS

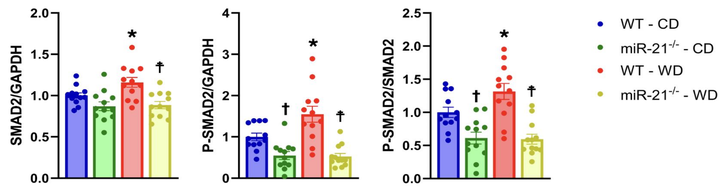

Figure 3. Western blot quantification of SMAD2 signaling proteins in wild-type (WT) and miR-21 knockout (miR-21$^{-/-}$) mice fed either a control diet (CD) or Western diet (WD). Protein expression of total SMAD2, phosphorylated SMAD2 (pSMAD2), and the pSMAD2/SMAD2 activation ratio were analyzed and normalized to GAPDH. Western diet increased SMAD2 phosphorylation in WT mice, while miR-21 deletion attenuated this response. Data are presented as mean ± SEM for n = 12 mice per group. *P<0.05 control vs. WT-WD group. †P<0.05 control vs. miR-21$^{-/-}$ CD. ǂP<0.05 WT-WD vs. miR-21$^{-/-}$ WD.

RESULTS

A.

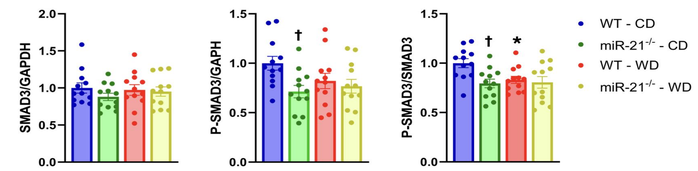

Figure 4. Western blot quantification of SMAD3 signaling proteins in wild-type (WT) and miR-21 knockout (miR-21$^{-/-}$) mice fed either a control diet (CD) or Western diet (WD). Protein expression of total SMAD3, phosphorylated SMAD3 (pSMAD3), and the pSMAD3/SMAD3 activation ratio were measured and normalized to GAPDH. MiR-21 deletion altered SMAD3 phosphorylation compared with WT mice, suggesting modulation of SMAD-dependent signaling pathways. Data are presented as mean ± SEM for n = 12 mice per group. *P<0.05 control vs. WT-WD group. †P<0.05 control vs. miR-21$^{-/-}$ CD.

A.

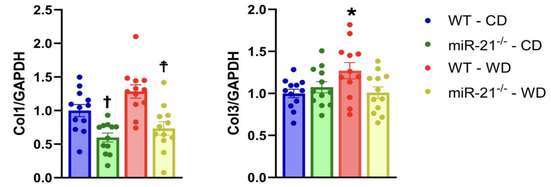

Figure 5. Western blot quantification of collagen proteins in wild-type (WT) and miR-21 knockout (miR-21$^{-/-}$) mice fed either a control diet (CD) or Western diet (WD). Protein expression of collagen type I (Col1) and collagen type III (Col3) was analyzed and normalized to GAPDH. Col1/GAPDH was significantly decreased in miR-21$^{-/-}$ CD compared with WT-CD, and this pattern persisted under Western diet conditions (WT-WD vs. miR-21$^{-/-}$ WD). Col3/GAPDH was significantly elevated in WT-WD compared with WT-CD, with no significant differences observed in miR-21$^{-/-}$ groups. Data are presented as mean ± SEM for n = 12 mice per group. *P<0.05 control vs. WT-WD group. †P<0.05 control vs. miR-21$^{-/-}$ CD. ǂP<0.05 WT-WD vs. miR-21$^{-/-}$ WD.

CONCLUSIONS

- Western diet increased SMAD2 phosphorylation in WT mice, indicating activation of profibrotic signaling pathways
- MicroRNA-21 deletion attenuated expression of proteins associated with SMAD2/3 signaling, suggesting miR-21 contributes to diet-induced activation of SMAD pathways
- MiR-21$^{-/-}$ mice have significantly lower collagen expression than wild type mice. Additionally, a western diet led to significant increases in collagen expression within wild type mice, which was not seen with MiR-21$^{-/-}$ mice

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