Collagen Peptide Inhibits Fibroblast Differentiation: A Potential Anti-Fibrotic Factor

Hanifah Hendricks, Kaitlin Whitehead, and Lisandra E. de Castro Brás

Hanifah Hendricks
Brody School of Medicine
Department of Physiology
East Carolina University
Greenville, North Carolina 27858
hendricksh19@students.ecu.edu

INTRODUCTION

Upon injury or disease, fibroblasts differentiate into myofibroblasts; these cells, which have a secretory and contractile phenotype, are the main source of fibrosis. Myofibroblasts have been shown to associate with adverse left ventricular (LV) myocardial remodeling leading to progressive loss of cardiac function. Matricryptin p1159, a biologically active collagen-derived peptide, has previously been shown to change fibroblast function and reduce fibrosis in a mouse model of adverse LV remodeling.

Accordingly, we hypothesized that p1159 reduces fibrosis by directly interacting with fibroblasts through integrins (Itg), a major family of matricryptin receptors. Our goals were to identify the mechanisms underlying p1159 modulation of cardiac fibroblast function.

MATERIALS & METHODS

Cell line: PromoCell line #C-12377, Human Cardiac Fibroblasts (HCF)

METHODS: Semi-confluent HCF cells in T75 flasks were:

- Starved overnight in low serum media (LSM)
- Treated with different conditions as in Table 1 (all solutions diluted in LSM, n=3/group)
- A group of cells were incubated with a neutralizing antibody against Itga4 (#NBP1-26661, Novus Biologicals; Itga4i) at 15 µg/mL for 2h. After this period, some of those cells had the media changed, were rinsed, and incubated with new LSM + p1159
- Cell lysates were collected for testing of expression of several markers of fibrosis
- RNA was extracted using a Pure Link RNA mini kit and quantified using the NanoDrop2000
- Reverse transcription was performed to generate cDNA
- Quantitative RT-PCR was performed for the genes:
  - αSMA, collagen-I, collagen-III, vimentin, Gapdh (housekeeping gene), and TGFβ (3 technical replicas/sample/gene)
- Data is displayed as $2^{-\Delta Ct}$ fold change to respective control (either LSM or LSM + Itga4i)

Flask #	Label
1A	LSM
1B	LSM
1C	LSM
2A	LSM + Itga4i
2B	LSM + Itga4i
2C	LSM + Itga4i
3A	LSM + 100nM pep
3B	LSM + 100nM pep
3C	LSM + 100nM pep
4A	LSM + 500nM pep
4B	LSM + 500nM pep
4C	LSM + 500nM pep
5A	LSM + Itga4i + 100nM pep
5B	LSM + Itga4i + 100nM pep
5C	LSM + Itga4i + 100nM pep
6A	LSM + Itga4i + 500nM pep
6B	LSM + Itga4i + 500nM pep
6C	LSM + Itga4i + 500nM pep

RESULTS

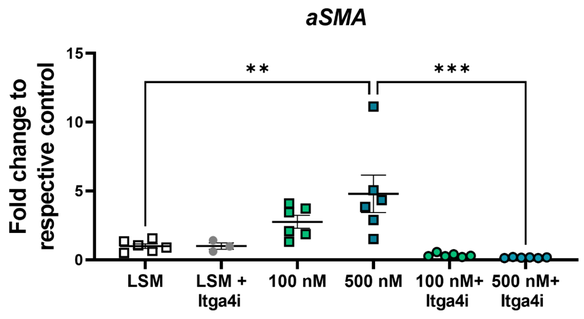

Figure 1- 500 nM of p1159 promoted expression of αSMA and this was dependent of Itga4.

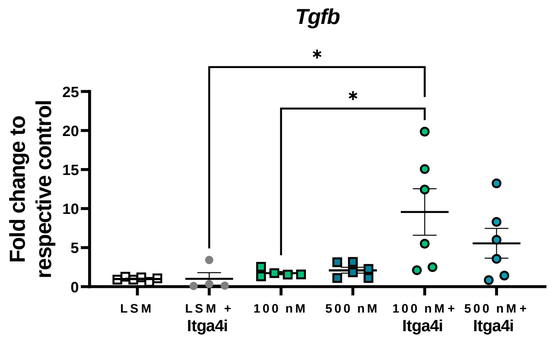

Figure 2- 100 nM of p1159 promoted expression of TGFβ when added in combination with Itga4 inhibitor.

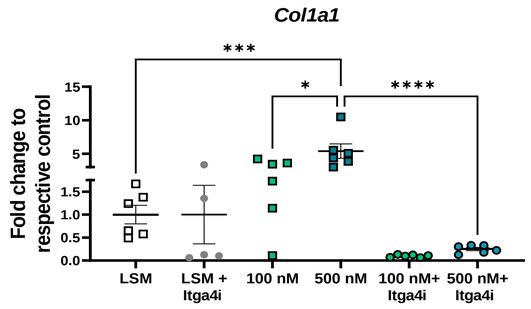

Figure 3- 500 nM of p1159 promoted expression of Col1a1 and this was dependent of Itga4.

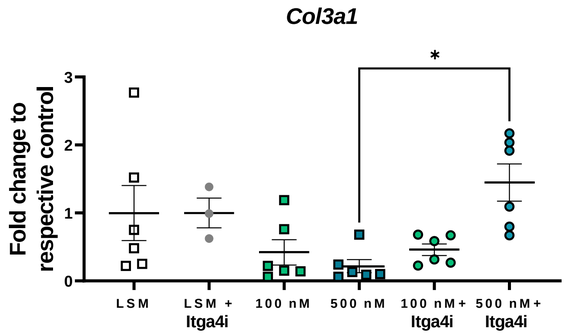

Figure 4- 500 nM of p1159 promoted expression of Col3a1 when added in combination with Itga4 inhibitor

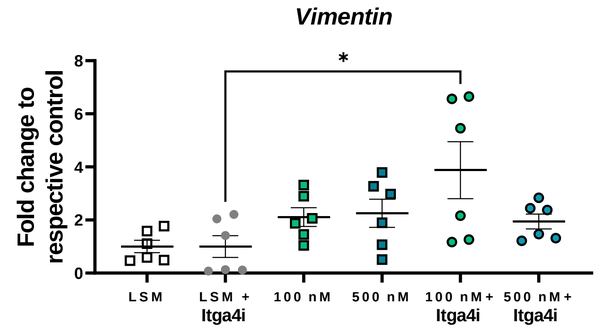

Figure 5- 100 nM of p1159 promoted expression of vimentin when added in combination with Itga4 inhibitor.

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DISCUSSION

Scar formation and fibrosis are primarily driven by inflammatory factors and mediated by differentiation of fibroblasts into myofibroblasts. A strong body of evidence associates myofibroblast actions with adverse myocardial remodeling [1-5]. The most prominent feature of myofibroblasts is their migratory and contractile phenotype, which results from the expression of contractile proteins such as α-smooth muscle actin (αSMA).

Previous experiments in the Brás lab support a role of p1159 in fibroblast differentiation into myofibroblast. During fibrosis, TGFβ and collagen-III are present early in the remodeling process, and expression of both was increased with the addition of peptide p1159 in combination with Itga4 inhibitor.

In our experiments to determine the connection between p1159 and Itgs, the highest dose of p1159 promoted expression of both collagen-I and αSMA and this was dependent of Itga4 (Figure 1 and Figure 3). This suggests that p1159 could potentially change fibroblast functions into a pro-fibrotic phenotype (contrary to our initial hypothesis). Although the matricryptin did not directly promote expression of collagen-III, TGFβ, and vimentin; when Itga4 was blocked, we observed an increase in collagen-III at 500nM of p1159, as well as TGFβ and vimentin at 100 nM p1159 (Figures 2, 4, and 5).

Itga4 is a known receptor for fibronectin, also an extracellular matrix protein that increases during fibrosis. There are no reports of Itga4 binding to collagen; this suggests that the bioactive cryptic sites that are exposed in matricryptins not only confer different functions to these peptides that differ from those of the parent molecules, but also bind to distinct receptors.

Overall, these data suggest that Itga4 is a receptor for p1159 in fibroblasts. However, it is possible that the peptide also binds to other receptors when Itag4 is not available, and this changes the expression of the pro-fibrotic molecules collagen-III, TGFβ, and αSMA. Future experiments need to be focused on p1159 effects on fibroblast phenotype.

ACKNOWLEDGEMENTS

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