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Stapled Peptide Inhibitors Targeting pre-miR-155 to Suppress miRNA Maturation

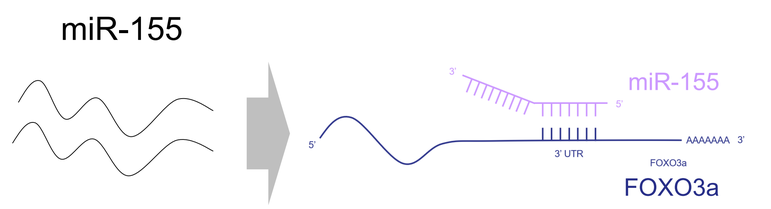
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Abstract

MicroRNA-155 (miR-155) is a well-established oncogenic microRNA, and inhibition of its maturation from precursor miR-155 (pre-miR-155) offers a potential strategy for modulating miR-155-driven signaling. Because pre-miR-155 contains a structured hairpin region involved in Dicer-mediated processing, it represents an attractive target for RNA-directed ligands. In this study, we investigated hydrocarbon-stapled peptides as modulators of pre-miR-155 maturation. Stapled analogues were designed from a previously reported pre-miR-155-binding peptide by introducing i, i + 4 hydrocarbon staples at selected positions predicted to avoid the RNA-recognition interface. Among the analogues, **S1** retained strong pre-miR-155 binding, inhibited Dicer-mediated cleavage, and showed improved serum stability and cellular uptake relative to the parent linear peptide. In cells, **S1** decreased mature miR-155 levels, increased pre-miR-155 accumulation, restored expression of the miR-155 target protein SOCS1, and reduced cell viability. These findings indicate that hydrocarbon stapling can improve the functional properties of RNA-targeting peptides when the staple is positioned to preserve the RNA-recognition interface. Overall, this work highlights stapled peptides as a promising modality for targeting structured RNA motifs involved in microRNA processing.

Introduction

How miR-155 Drives Tumor Progression



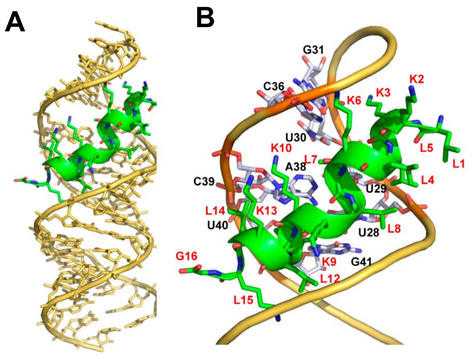
Overexpressed in cancer cell → Suppress tumor suppressor genes → Inhibit apoptosis → Tumor progression

Structure Basis for Targeting pre-miR-155

N-terminal **C-terminal**

159: LKKLLKLLKKWLK

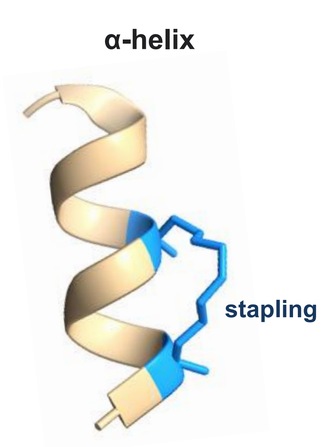
A B



"Peptide 159, a pre-miR-155 Dicer cleavage site-binding peptide, was used as a template for inhibitor development."

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Advantage of stapled peptide



Stablize α-helix structure → Binding affinity↑ → Cell Penetration↑ → Protease resistance↑ → Drug property↑

Fig. 1. Molecular model of peptide 159 bound to pre-miR-155. (A) Overall structure of the peptide 159–pre-miR-155 complex. (B) Close-up view showing peptide binding within the major groove of the apical stem-loop.

Binding assay

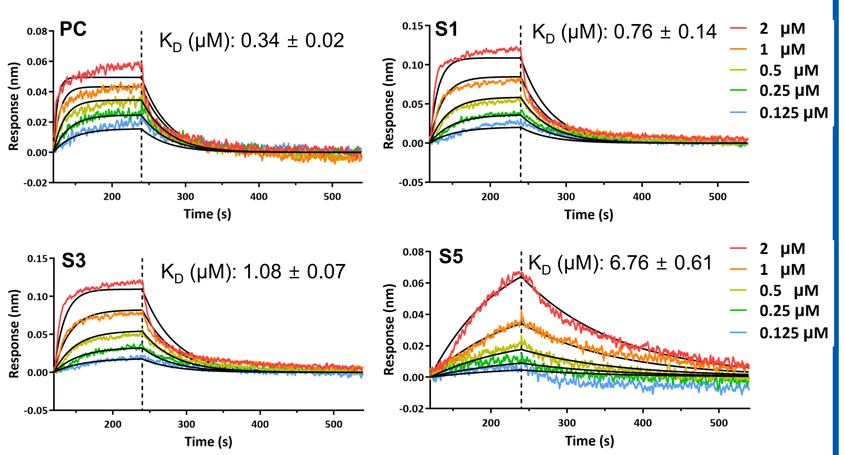
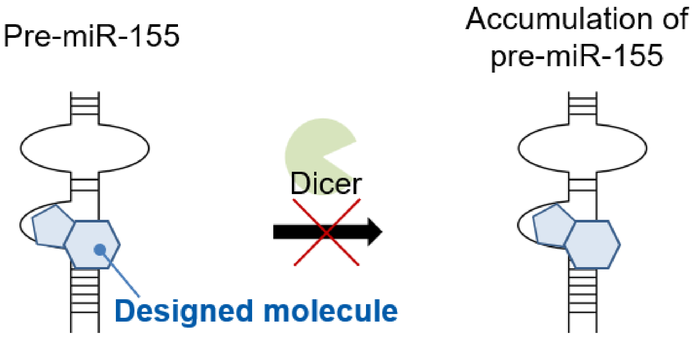


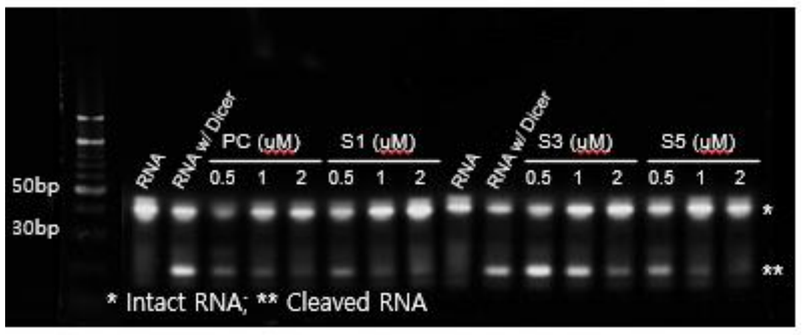
Fig. 3. BLI binding curves of fluorescein-labeled peptide 159 and its stapled analogs (S1, S3, and S5) with pre-miR-155. K_D values were determined from the binding curves.

Dicer Inhibition Assay

A. Experimental Design



B.



C.

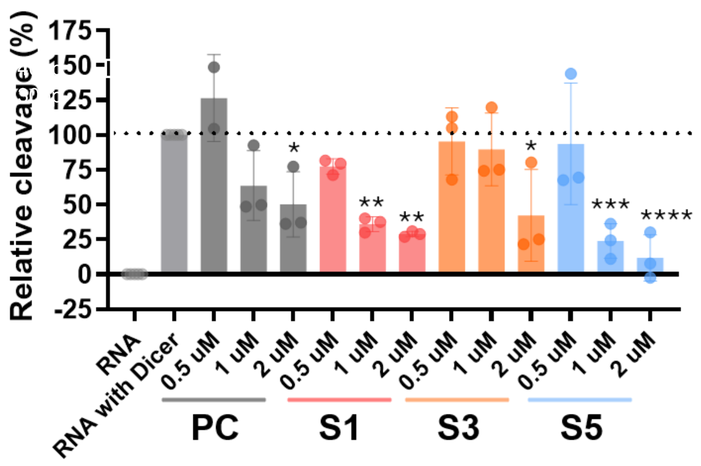


Fig. 4. (A) Experimental design of dicer inhibition assay. (B) Fluorescence-labeled pre-miR-155 was treated with peptide **159** (positive control) and synthesized peptides **S1**, **S3**, and **S5** at concentration 1μM. After incubation with Dicer at 37 °C for 2 hours, samples were analyzed by 8% PAGE. (C) Quantification of Dicer-mediated pre-miR-155 cleavage relative to the RNA with Dicer control (100%).

Cell Penetration Efficiency

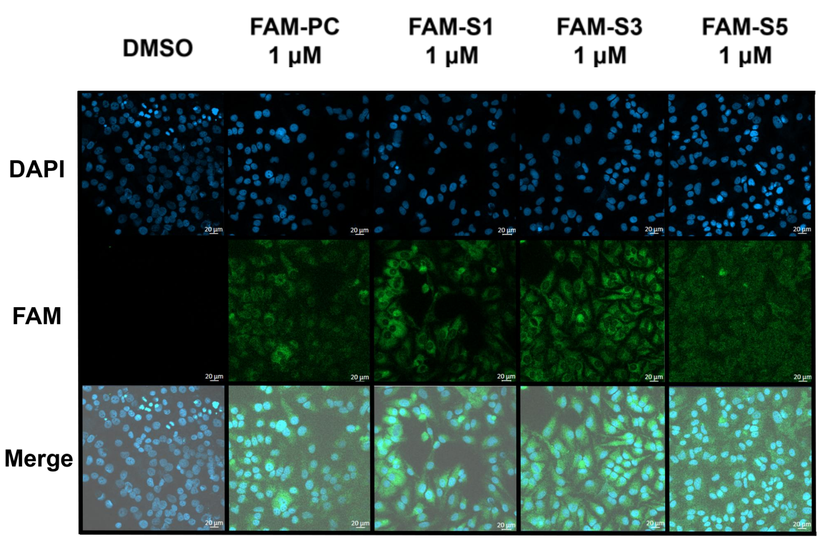


Fig. 5. Cellular uptake of FAM-labeled peptide 159 (PC) and its stapled analogs (S1, S3, and S5) in HeLa cells. Cells were treated with each peptide at 1 μM for 12 h, and cellular uptake was visualized by confocal microscopy.

RT-qPCR & Western Blot

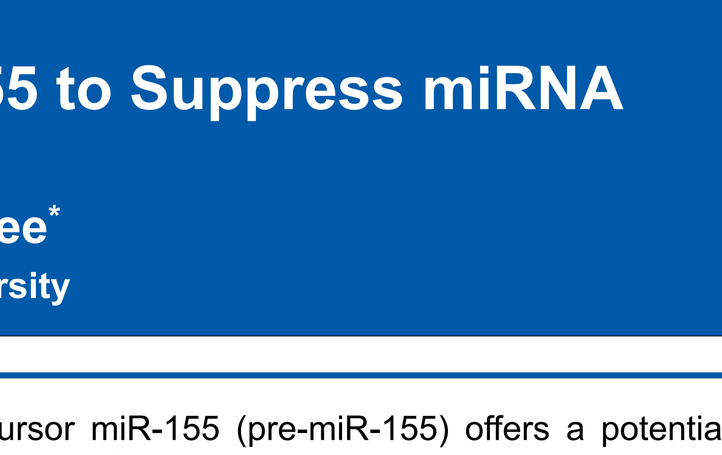


Fig. 6. (A) RT-qPCR analysis of relative miR-155 levels normalized to GAPDH for respectively.
(B) Representative Western blots and corresponding quantification of SOCS1 protein levels normalized to α - actinin. Data are presented as mean ± SD (n = 3).

Effect of Dicer inhibitor in Apoptosis

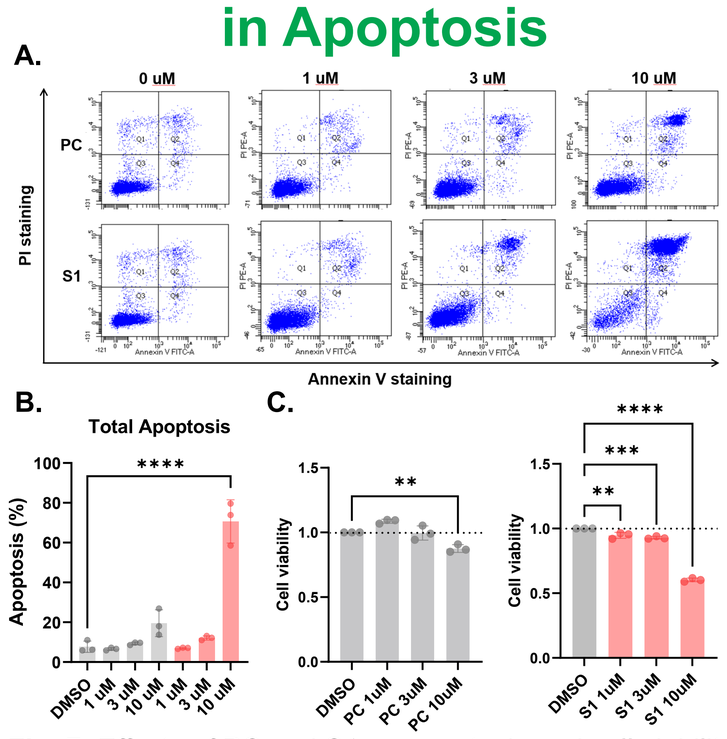


Fig. 7. Effects of PC and S1 on apoptosis and cell viability. (A) Annexin V/PI analysis following treatment with PC or S1. (B) Quantification of total apoptosis. (C) Cell viability at the indicated peptide concentrations.

Conclusions

Stapling of peptide **159** generated conformationally constrained analogs with distinct RNA-binding and cellular properties. Among the stapled peptides, S1 and S3 showed favorable binding to pre-miR-155 and efficient cellular uptake, whereas S5 exhibited relatively weak binding and uptake. The stapled peptides modulated Dicer-mediated pre-miR-155 processing, resulting in increased pre-miR-155 accumulation and reduced mature miR-155 levels in cells. In particular, S1 induced dose-dependent apoptosis accompanied by reduced cell viability. Collectively, these results demonstrate that peptide stapling can enhance the functional properties of RNA-targeting peptides, supporting their potential as a strategy for modulating miRNA maturation.

Reference

[1] J Pai et al., "Screening of pre-miRNA-155 Binding Peptides for Apoptosis-Inducing Activity Using Peptide Microarrays", J. Am. Chem. Soc. **2016**, *138*, 857–867.

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