Inhibiting mechanotransduction as a novel approach for cancer therapy

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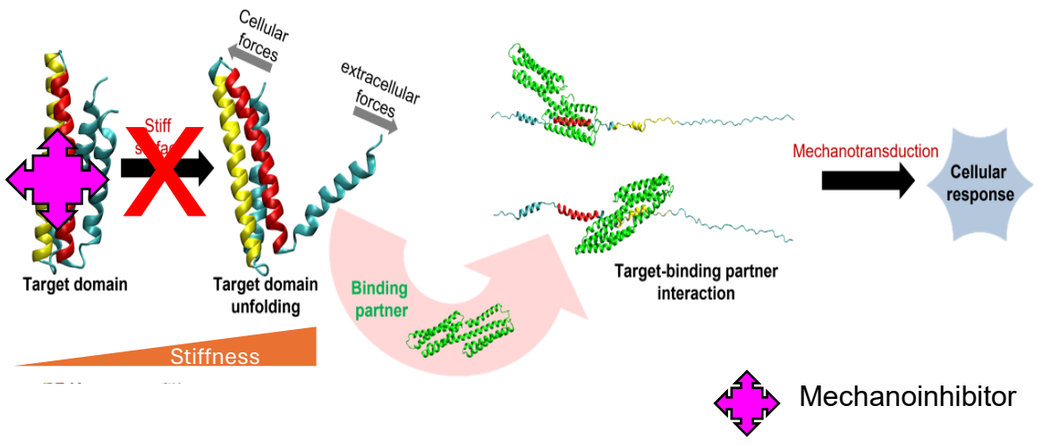
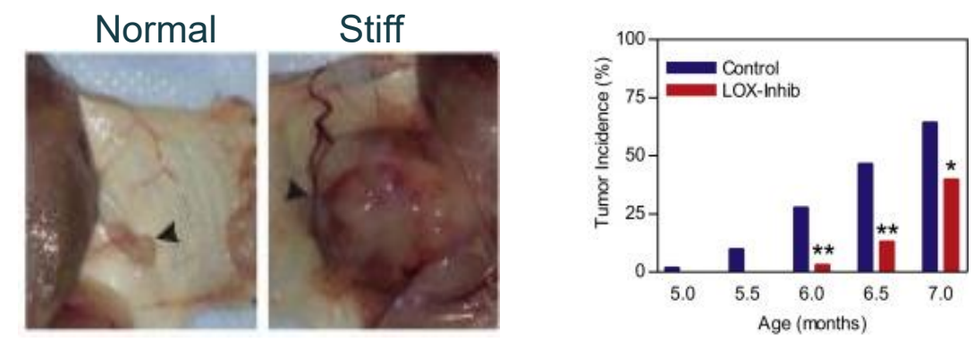
Graphical Abstract



Hypothesis: a mechanoinhibitor (magenta inset) bind to the target protein, mechanically reinforcing its stability. This hinders the target unfolding for cells in stiff substrates, subsequently blocking mechanotransduction events that promote tumor progression.

Background

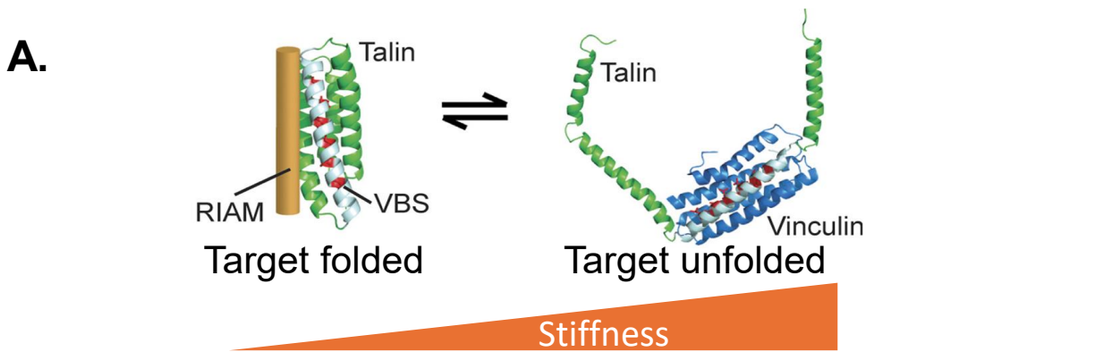


Increasing/decreasing stiffness promotes/inhibits tumour progression

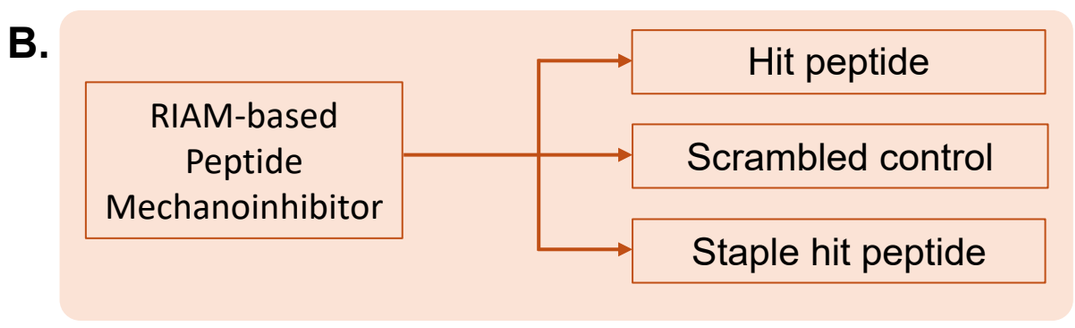
Levental *et al., Cell* 2009

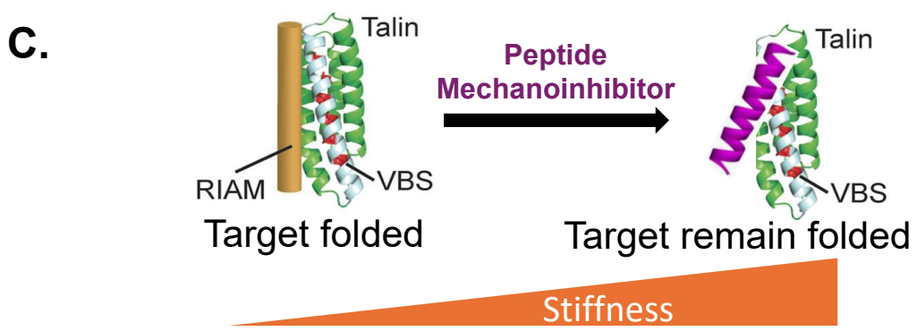
- Currently, there are **no compounds or drugs** available that specifically block mechanotransduction.

Methodology



Benjamin T Goult, *et. al.* J Biol Chem. 2013



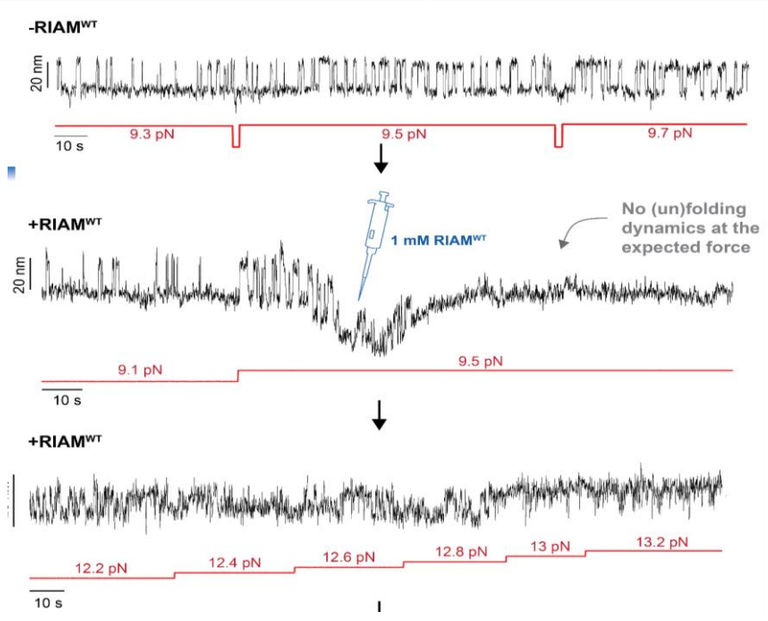


A. Target binds to RIAM when folded but unfolds in cells on stiff substrates, binding to Vinculin. **B.** Design of mechanoinhibitor peptide based on RIAM. **C.** Peptide mechanoinhibitor (magenta) binds to target, mechanically reinforcing its stability and target remain folded on stiff substrates.

Results

In vitro validation

RIAM-based Peptide Mechanoinhibitor	Thermal stability measurement		Affinity (Kd) measurement by Surface plasma resonance (SPR)	Mechanical stability measurement by magnetic tweezer (pN force shift)
	Thermal shift assay (ΔTm in °C)	Circular Dichroism (ΔTm in °C)		
Hit peptide	3	1	~110 µM	~3
Staple hit peptide	7	5.5	~50 µM	~3
Scrambled control	0	N/A	No binding	N/A

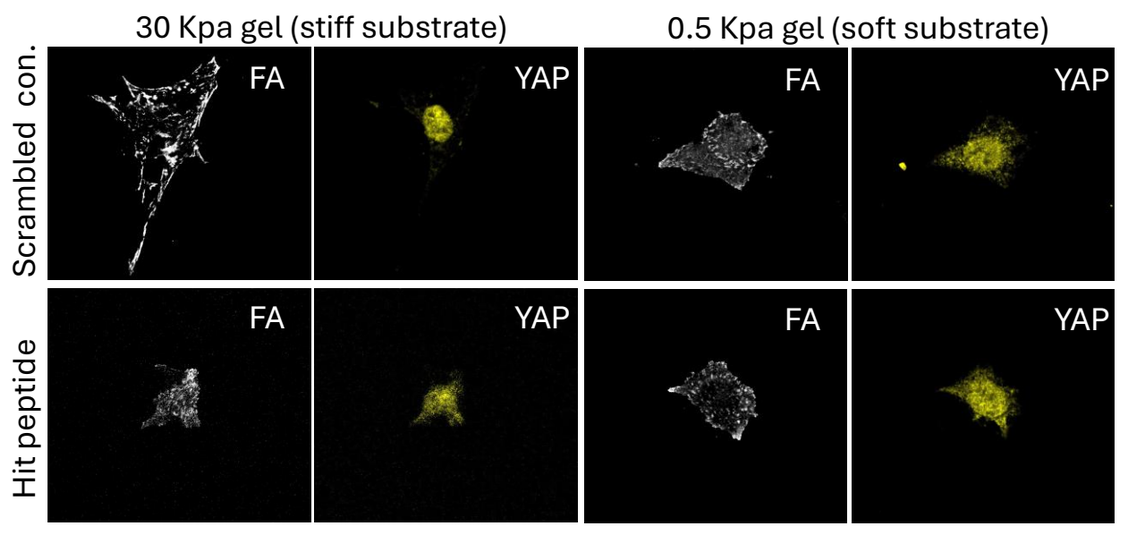


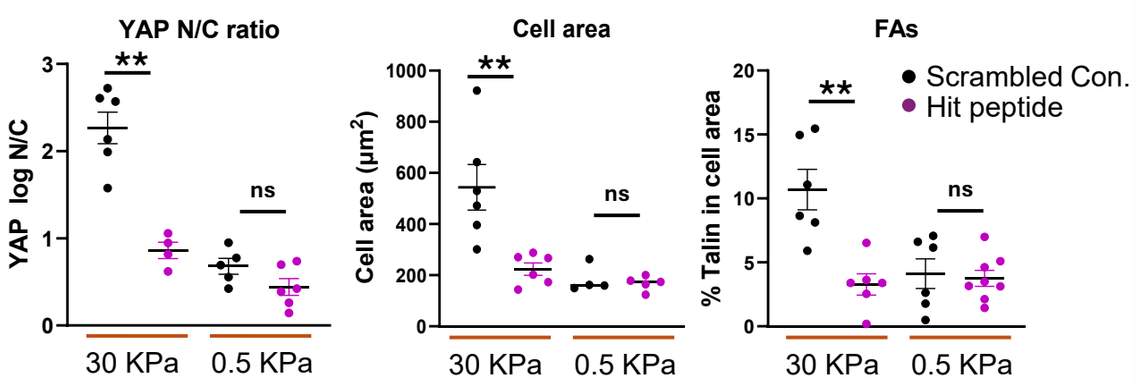
Magnetic tweezer data

Top panel. without hit peptide the target protein unfolds around 9 pN

Middle & Bottom panel. Upon injection of peptide hit, unfolding dynamics instead happens at higher force of 13 pN

In Cellulo validation





Representative images of fibroblast cells on stiff (30 KPa) and soft (0.5 KPa) substrates expressing either hit peptide or scrambled control. YAP staining (yellow), and Talin protein (grey) for Focal Adhesions (FAs)

Bottom panel. Quantitative analysis of the above images. Each dot indicates a cell, mean with std. error is shown in the plots. ** indicates p value <0.01

Take home message

- Staple hit peptide is able to bind and stabilize the target most effectively *in vitro*.
- Hit peptide exhibits decreased cell area, FAs and YAP Nuclear to cytoplasmic (N/C) ratio compared to scrambled control specifically on stiff substrates, indicating specific inhibition of target-induced mechanotransduction.

Upcoming work: Evaluation of anti-tumor and metastatic inhibition ability of the peptide hits in pancreatic cancer mouse model to establish *in vivo* proof-of concept

ACCIÓ Tecniospring INDUSTRY
Generalitat de Catalunya Government of Catalonia

The project leading to these results has received funding from the European Union's Horizon 2020 research and innovation programme under Marie Skłodowska-Curie grant agreement No.801342 (Tecniospring INDUSTRY) and the Government of Catalonia's Agency for Business Competitiveness (ACCIÓ).

Funded by the European Union

European Research Council
Established by the European Commission

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