The Impact of Matrix Stiffness and O-GlcNAcylation on YAP Nuclear Localization and Matrix Deposition in Mesenchymal Stem Cells

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Introduction

- Mechanotransduction is the process in which cells convert physical cues from their microenvironments into biochemical signals that modulate cell activity and fate[1]
- Yes-associated protein (YAP) is an important transcriptional co-activator in mechanotransduction[1,2]
- **The molecular mechanism by which YAP localizes to the nucleus is poorly understood (Fig. 1)**

- Recent studies in cancer biology, have shown that O-linked β-*N*-acetylglucosamine (O-GlcNAc) modifications on YAP are necessary for YAP nuclear localization[3,4]
- O-GlcNAcylation is a common post-translational modification for cytoplasmic and nuclear proteins[5]

- **We hypothesized that the addition of O-GlcNAc groups on YAP by OGT promotes nuclear translocation in a mechanotransductive setting.**

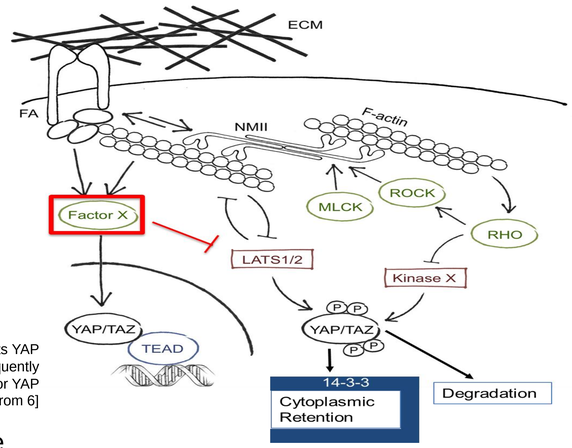


Figure 1: Working model for YAP regulation. Activation of Rho signaling inhibits YAP antagonists, LATS1/2 and Kinase X. YAP is inhibited by phosphorylation and is subsequently degraded or cytoplasmically retained. Factor X could be acting as a direct partner for YAP nuclear localization or acting as another inhibitor of LATS1/2 [modified from 6]

The timing and amount ECM deposition defines tissue maturation and structure-function relationships, and is uniquely tuned to enable musculoskeletal tissue function.

- Matrix stiffness increases mechanobiologic signaling (e.g., YAP nuclear localization) in MSCs[7]
- **The link between mechanosensing and nascent matrix production has not yet been explored.**
- **To address this, we utilized tunable biomaterial substrates (norbornene modified HA hydrogels) to evaluate substrate stiffness and ECM formation.**

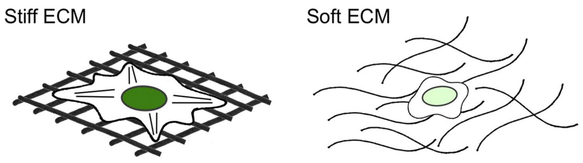


Figure 2: Model depicting the relationship between substrate stiffness and cell spreading. Cells seeded onto stiffer gels spread more than cells on softer substrates. [Adapted from 6].

Methods

Hydrogel fabrication: Fibronectin-coated polyacrylamide (PA) gels were produced as in [8]. RGD-modified NorHA hydrogels were produced as in [9].

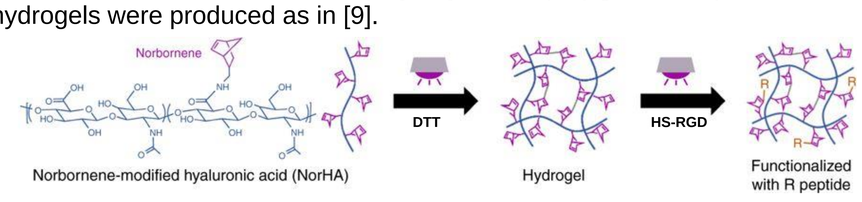
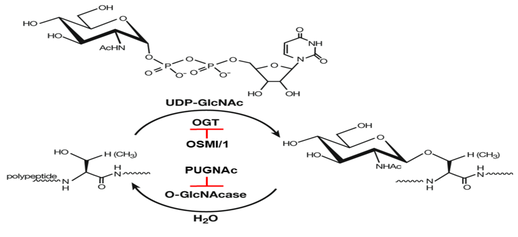


Figure 3: Schematic of NorHA structure, crosslinking, and functionalization with ligands. NorHA is crosslinked with Irgacure under UV light with DTT as a crosslinker and is concurrently functionalized with a thiolated RGD peptide [adapted from 9].



Pharmacological inhibition: OGT was inhibited with OSMI-1 (at 50mM). Cell contractility was inhibited with Y-27632 (at 10uM) or increased with LPA (at 50uM).

Figure 4: Kinetics of O-GlcNAc addition and removal by OGT and O-GlcNAcase, respectively. OSMI-1 inhibits OGT, while PUGNAc inhibits O-GlcNAcase.

AHA labeling: To identify nascent matrix, cells were cultured in media containing AHA, a methionine substitute. DBCO-488 was used to label all proteins containing AHA, with labeling performed on live cells prior to fixation. A membrane stain was used to create a cell mask in order to identify extracellular proteins.

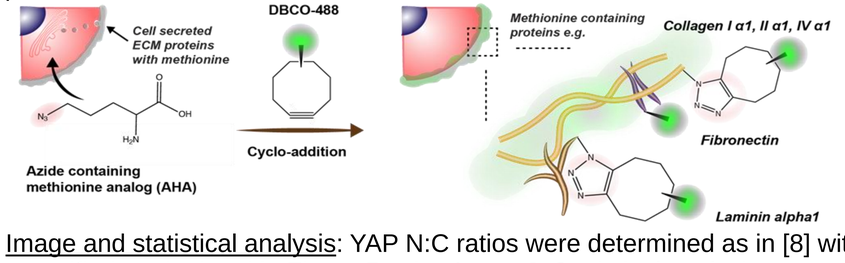


Figure 4: Model showing how secreted proteins are labeled. AHA is used as a methionine analog and proteins that have incorporated AHA are visualized with DBCO-488 using cyclo-addition.

Image and statistical analysis: YAP N:C ratios were determined as in [8] with ImageJ. Nascent matrix was quantified as depicted in Figure 5 using ImageJ. Statistical analysis was performed by one-way ANOVA with Tukey's post-hoc tests.

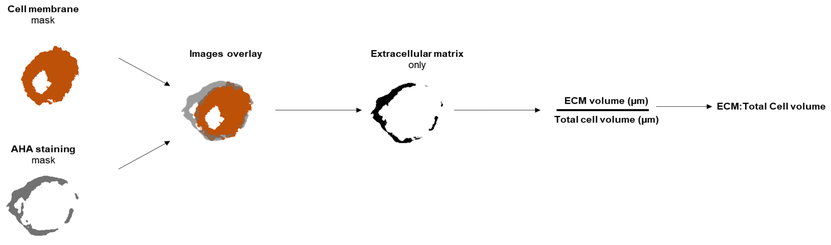


Figure 5: Schematic for method of determining extracellular protein amount. Cellmask was used to stain the membrane to eliminate membrane bound proteins, DBCO-488 was used to identify secreted proteins that had incorporated the AHA. These two images were overlayed and subtracted to yield a resultant image that contained non-membrane associated proteins. ECM volume was normalized by dividing by the total cell volume.

Results

Inhibition of OGT on stiff substrates decreases YAP N:C ratios

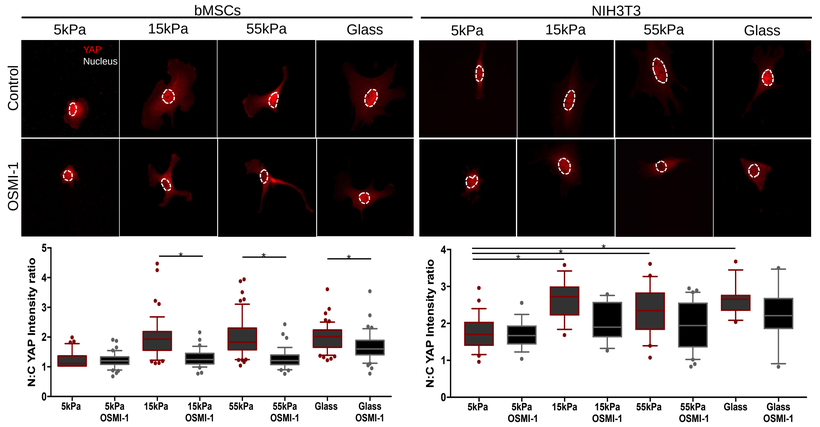


Figure 6: OSMI-1 treatment reduces YAP N:C ratios to levels seen on soft substrates. For bMSCs, N=3 replicates, n=39-53 cells per condition. For 3T3 cells, N=2 replicates, n=14-30 cells per condition. *p<0.05

- Both cell types showed increased YAP N:C with increased substrate stiffness
- OGT inhibition resulted in lower YAP N:C ratios on stiff substrates, matching levels seen on soft substrates (5kPa)
- OGT inhibition did not alter YAP N:C ratios on 5kPa PA gels, where the ratio was already low

Inhibition of OGT decreases the number of focal adhesions

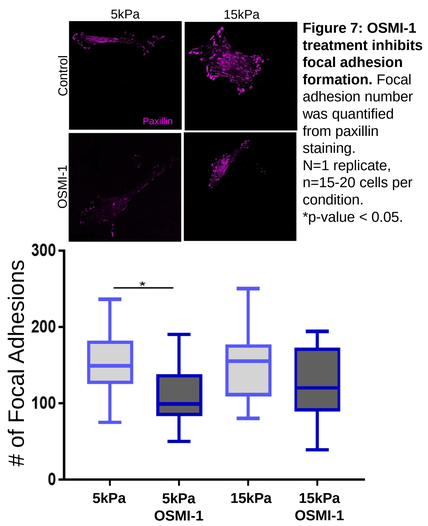


Figure 7: OSMI-1 treatment inhibits focal adhesion formation. Focal adhesion number was quantified from paxillin staining. N=1 replicate, n=15-20 cells per condition. *p-value < 0.05.

- OGT inhibition reduced the number of focal adhesions on 5kPa NorHA gels

Substrate stiffness influences early, but not late nascent matrix deposition

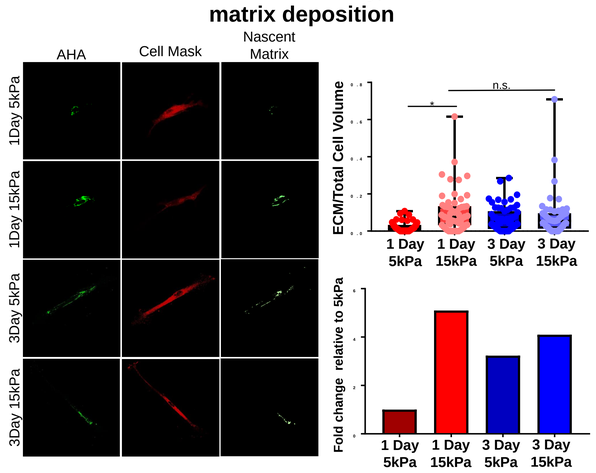


Figure 8: Substrate stiffness increases nascent matrix deposition. hMSCs were seeded onto RGD-modified NorHA gels for the times indicated. N=3 replicates, n=20-25 cells per condition. *p<0.05

- Cells on 15kPa NorHA gels deposited more nascent matrix than on 5kPa NorHA gels after 1 day
- After 3 days, matrix deposition was similar, regardless of substrate stiffness

Cell contractility regulates nascent matrix production

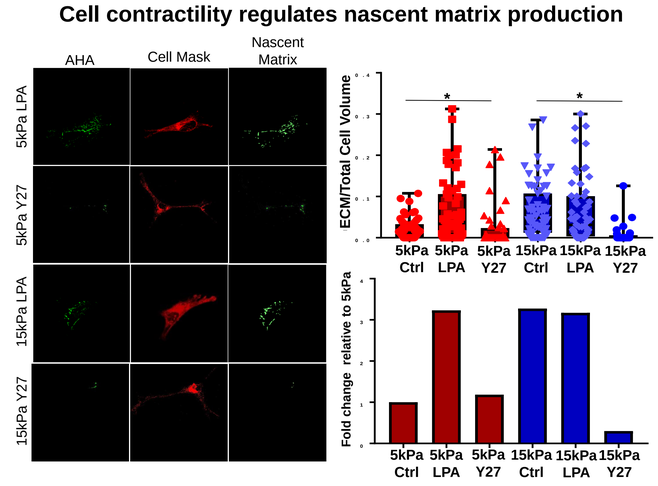


Figure 9: Contractility regulates nascent matrix production. N=3 replicates, n=20-25 cells per condition. *p-value < 0.05.

- Nascent matrix deposition is rescued on soft substrates by increasing contractility
- On stiffer substrates, nascent matrix deposition is abrogated by reducing contractility

Discussion

- Glucose availability is the limiting reagent in O-GlcNAc modifications and tissue possess unique glucose availability[5].
  - It could be that cells modulate mechanotransduction through metabolism.
- Further work will be done to:
  - Evaluate the consequences of early vs. late matrix deposition in MSC differentiation.
  - Elucidate possible cross-talk mechanisms between O-GlcNAc mediated mechanotransduction and nascent matrix production.

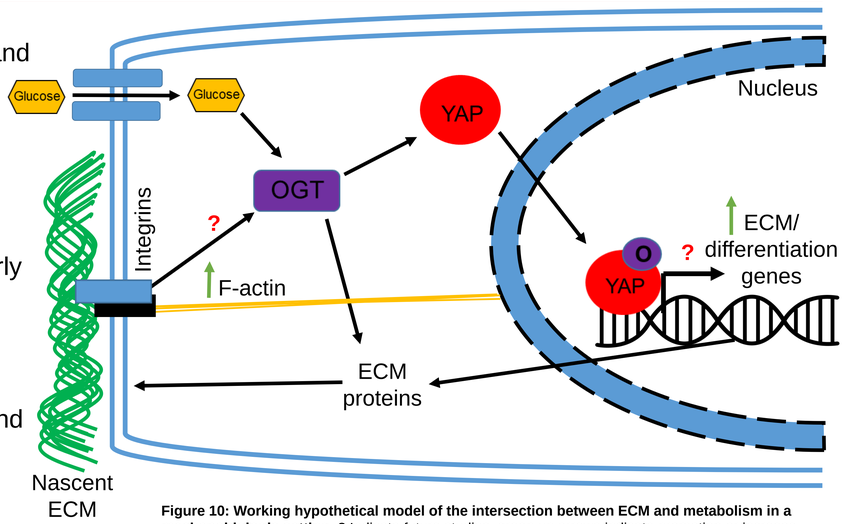


Figure 10: Working hypothetical model of the intersection between ECM and metabolism in a mechanobiologic setting. ? Indicate future studies, green up arrows indicate promotion or increase. Molecules not to scale

References

[1] Dupont, S. et al., *Nature*, 7350: 179–183, 2011. **[2]** Halder, G. et al.,. *Nat. Rev. Mol. Cell Biol.*, 9: 591–600,2012. **[3]** Zhang, X. et al., *Nat. Commun.*, 8: 15280, 2017. **[4]** Peng, C. et al., *Mol. Cell*, 3:591-604, 2017. **[5]** Hart G. et al., Essentials of Glycobiology. 2nd edition. Cold Spring Harbor Laboratory Press, 2009. **[6]** Dupont, S. *Exp. Cell Res.*, 1: 42–53, 2016. **[7]** Smith et al. *Physiology.*, 33(1): 16–25., 2018. **[8]** Dupont et al., *Nature* 474(7350):179-83 2011. **[9]** Vega et al. *Nature Comm.* 9;9(1):614 2018.

Acknowledgements

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