

BM2023 Assignment 2

Find 5 research or clinical papers on mechanotransduction of stem cells and their differentiation. Find the data relevant to RAP2 in cartilage or respective genes and tissues. Summarize the data from the abstract to make a short report for each gene function based on literature search. Find the data and report about the data headings from the database. Find the related information and highlight the ONE THING you found important. Take the citation for your future reference and put it in the word file of your report.

Stem cells are a typical model system in the mechanotransduction field; it has been shown that differentiation of stem cells into distinct cell fates is dictated by the physical features of the cellular microenvironment. YAP/TAZ is a key regulator of mechanical properties of the stem cell microenvironment. RAP2 and YAP/TAZ play pivotal roles in mechano-regulated transcription. Various biophysical stimuli from external forces, such as cyclic stretching, shear stress and acoustic tweezing, also modulate stem cell fate via YAP/TAZ. For example, fluid shear stress serves an important role in the differentiation of embryonic and mesenchymal stem cells (MSCs).

1. From the abstract of 'RAP2 mediates mechanoresponses of the Hippo pathway' (Meng, Z., Qiu, Y., Lin, K.C. *et al.* RAP2 mediates mechanoresponses of the Hippo pathway. *Nature* 560, 655–660 (2018). <https://doi.org/10.1038/s41586-018-0444-0>), we learn that extracellular matrix (ECM) and neighboring cells that surround mammalian cells and provide them with structural support and mechanical cues that influence diverse biological processes. Mechanical cues regulate Hippo pathway effectors YAP (also known as YAP1) and TAZ (also known as WWTR1) and mediate cellular responses to ECM (matrix) stiffness. In this study, the Ras-related GTPase RAP2 was identified as a key

intracellular signal transducer that, through YAP and TAZ, relays ECM rigidity signals to control mechanosensitive cellular activities. RAP2 is activated by low ECM stiffness. Deletion of RAP2 blocks the regulation of YAP and TAZ by stiffness signals and hence, promotes aberrant cell growth. Mechanically, ECM stiffness affects levels of phosphatidylinositol 4,5-bisphosphate and phosphatidic acid, by acting via phospholipase C γ 1 (PLC γ 1), which activates RAP2 via PDZGEF1 (also known as RAPGEF2) and PDZGEF2 (also known as RAPGEF6). At low matrix stiffness, active RAP2 binds to and promotes MAP4K4, MAP4K6, MAP4K7 and ARHGAP29, which activates LATS1 and LATS2 and inhibits YAP and TAZ. As deletion of YAP and TAZ abolishes the extracellular matrix stiffness-responsive transcriptome, RAP2, YAP and TAZ have crucial roles in mechanoregulated transcription. These findings establish a mechanosignalling pathway from matrix stiffness to the nucleus, by demonstrating the role of RAP2 as a molecular switch in mechanotransduction. This study reveals that **RAP2 is an intracellular mechanotransducer**, which through the Hippo pathway, relays extracellular mechanical signals to transcriptional regulation.

2. The paper 'Controllable ligand spacing stimulates cellular mechanotransduction and promotes stem cell osteogenic differentiation on soft hydrogel' (Man Zhang, Qian Sun, Yiling Liu, Zhiqin Chu, Leixiao Yu, Yong Hou, Heemin Kang, Qiang Wei, Weifeng Zhao, Joachim P. Spatz, Changsheng Zhao, Elisabetta A. Cavalcanti-Adam, 'Controllable ligand spacing stimulates cellular mechanotransduction and promotes stem cell osteogenic differentiation on soft hydrogels', *Biomaterials*,

Volume 268, 2021, 120543, ISSN 0142-9612, <https://doi.org/10.1016/j.biomaterials.2020.120543>.) briefly explains that hydrogels with adjustable mechanical characteristics have made it incredibly easy to control stem cell differentiation. Mesenchymal stem cells must typically be exposed to osteoid hydrogels (about 30–40 kPa or greater stiffness) or higher in order to be induced to differentiate into osteoblasts (MSCs). Due to limited cellular mechanotransduction and low levels of external mechanical stimulation, it is still challenging to produce the same differentiation on very soft hydrogels. Here, we use quasi-hexagonally ordered nanopatterns to alter cellular spatial sensing of integrin-adhesive ligands in order to enhance cell mechanosensing on hydrogels with low rigidity (about 3 kPa). It has been demonstrated that the increasing interligand spacing controls actomyosin force loading to attract more integrins on soft hydrogels. As a result, it stimulates mechanotransduction and encourages MSCs to differentiate into osteoblasts on soft hydrogels at a rate equivalent to that seen in osteoid rigidity. The creation of biomaterials and tissue scaffolds for regenerative treatments is now open to new possibilities thanks to this work. Many mechanosensitive genes can have their expression regulated by the transcriptional regulators YAP/TAZ. YAP/TAZ distribution inside cells is influenced by cytoskeleton tension and cell nuclear mechanics. By the control of RAP2, the ARID1A-containing SWI/SNF complex, and the tension-stretched nuclear pore opening, intracellular tension causes the movement of YAP/TAZ from the cytoplasm to the nucleus. Generally, nuclear localization of YAP/TAZ is only seen in cells on rigid substrates.

YAP immunostaining was used to look into the subcellular localization of YAP/TAZ in this instance. As predicted, when the ligand distance on soft hydrogels was 30 nm, YAP was mostly dispersed in the cytoplasm, but as the distance grew, it was increasingly concentrated in the cell nucleus. In stiff hydrogels, on the other hand, an increase in ligand distance resulted in YAP deactivation from the nucleus. As a result, ligand spacing and substrate stiffness worked together to synergistically influence cell transcriptional activity.

3. From the paper 'Matrix mechanotransduction mediated by thrombospondin-1/integrin/YAP in the vascular remodeling' (Yoshito Yamashiro, Bui Quoc Thang, Karina Ramirez, Seung Jae Shin, Tomohiro Kohata, Shigeaki Ohata, Tram Anh Vu Nguyen, Sumio Ohtsuki, Kazuaki Nagayama, and Hiromi Yanagisaw, 'Matrix mechanotransduction mediated by thrombospondin-1/integrin/YAP in the vascular remodeling, April 22, 2020, *Proceedings of the National Academy of Sciences of the United States of America*, National Academy of Sciences, 117 (18) 9896-9905, <https://doi.org/10.1073/pnas.1919702117>), we learn that mechanical cues, that are initiated by the extracellular matrix (ECM), activate intracellular signaling through matrix–cell interactions. Additional mechanical cues, in blood vessels, derived from the pulsatile blood flow and pressure play a crucial role in disease development and homeostasis. The nature of the mechanical cues from the extracellular matrix and their interaction with the mechanical microenvironment in large blood vessels to preserve the vessel wall's integrity are not fully understood. In this study, thrombospondin-1 (Thbs1), a matricellular protein, was recognised to be an extracellular mediator of extracellular matrix mechanotransduction that acts

through integrin $\alpha\beta 1$ to establish focal adhesions and helps promote nuclear shuttling of YAP (Yes-associated protein) in response to the high strain of cyclic stretch. The cyclic stretch causes Thbs1 to be secreted, which then binds to integrin $\alpha\beta 1$ and helps the FA–actin complex mature. This, in turn, mediates nuclear shuttling of YAP by inactivating Rap2 and the Hippo pathway. The alteration of actin fibers does not affect the activation of Thbs1-mediated YAP. The activation of Thbs1-mediated YAP depends on the Hippo pathway and the small GTPase Rap2. Inhibition of Thbs1/integrin $\beta 1$ /YAP signaling occurred when Thbs1 was deleted in mice, thus causing maladaptive remodeling of the aorta as a consequence of pressure overload and inhibition of the formation of neointima upon the ligation of carotid artery, hence, context-dependent effects are exerted on the vessel wall. Thus, a mechanism of matrix mechanotransduction centered on Thbs1, that during vascular remodeling, connects mechanical stimuli to YAP signaling in vivo was proposed in this study.

4. From the research article ‘Loss of RAP2A Aggravates Cartilage Degradation in TMJOA via YAP Signaling’ (Qi H, Zhang Y, Xu L, et al. Loss of RAP2A Aggravates Cartilage Degradation in TMJOA via YAP Signaling. *Journal of Dental Research*. 2023;102(3):302-312.doi:[10.1177/00220345221132213](https://doi.org/10.1177/00220345221132213)), we learn that abnormal stress loading is a significant factor in the development of TMJOA (Temporomandibular joint osteoarthritis). So far, investigations conducted have not found a functional molecule that converts physical stress into biological or biochemical signals in chondrocytes as a response to severe mechanical stress. Protein connected to Ras The Hippo/Yes-associated protein (YAP) pathway is a molecular

switch that transmits signals about extracellular matrix rigidity through Rap-2a (RAP2A). In the current work, RAP2A was found to be decreased in unilateral anterior crossbite-induced TMJ OA animals with cartilage degeneration as well as in conditional RAP2A deletion mice with significant cartilage matrix degeneration and TMJ OA development. The Hippo/YAP pathway was directly controlled by RAP2A in chondrocytes in response to matrix stiffness, and RAP2A/Hippo/YAP were essential for a phenotypic flip in chondrocytes as well as matrix formation. RAP2A deficiency harmed cartilage.

5. From the paper 'A spatial model of YAP/TAZ signaling reveals how stiffness, dimensionality, and shape contribute to emergent outcomes' (Kiersten Elizabeth Scott, Stephanie I. Fraley, and Padmini Rangamani, 'A spatial model of YAP/TAZ signaling reveals how stiffness, dimensionality, and shape contribute to emergent outcomes', May 14 2021, *Proceedings of the National Academy of Sciences*, National Academy of Sciences, 118 (20) e2021571118, <https://doi.org/10.1073/pnas.2021571118>), we learn that in order to carry out its duties, YAP/TAZ must move from the cytoplasm to the nucleus in response to a variety of physical signals. It is a master regulator of mechanotransduction. Input signals for YAP/TAZ include substrate stiffness, substrate dimensionality, and cell shape. This pathway controls vital cellular processes as well as tissue homeostasis. Uncertainty persists, however, regarding the relative contributions of each biophysical signal and the methods by which they cooperatively control YAP/TAZ in actual tissue microenvironments that offer multiplexed input signals. For instance, YAP/TAZ nuclear localization in basic two-dimensional cultures substantially corresponds with

substrate stiffness, yet in three-dimensional (3D) settings, YAP/TAZ translocation can either increase or decrease with stiffness or remain constant. To enable quantitative examination of the interactions between substrate stiffness, substrate dimensionality, and cell shape, we create a spatial model of YAP/TAZ translocation in this study. The model extends beyond the data currently available to predict that increasing substrate activation area through changes in culture dimensionality, while conserving cell volume, forces distinct shape changes that have a nonlinear effect on YAP/TAZ nuclear localization. The model couples cytosolic stiffness to nuclear mechanics to replicate existing experimental trends. Additionally, contrary tendencies in YAP/TAZ nuclear localization in 3D culture may be explained by changes in substrate activation area against total membrane area. Based on this multiscale analysis of the various YAP/TAZ nuclear translocation system characteristics, we hypothesise that a cell's ability to read its surroundings is a complex information transfer function involving a number of mechanical and biochemical variables. These predictions highlight a few cellular and tissue engineering design tenets for YAP/TAZ mechanotransduction.

Titles and Citations of the papers:

¹RAP2 Mediates Mechano-responses of Hippo Pathway (Meng, Z., Qiu, Y., Lin, K.C. *et al.* RAP2 mediates mechanoresponses of the Hippo pathway. *Nature* 560, 655–660 (2018). <https://doi.org/10.1038/s41586-018-0444-0>)

²Controllable ligand spacing stimulates cellular mechanotransduction and promotes stem cell osteogenic differentiation on soft hydrogel (Man Zhang, Qian Sun, Yiling Liu, Zhiqin Chu, Leixiao Yu, Yong Hou, Heemin Kang, Qiang Wei, Weifeng Zhao, Joachim P. Spatz, Changsheng Zhao, Elisabetta A. Cavalcanti-Adam, 'Controllable ligand spacing stimulates cellular mechanotransduction and promotes stem cell osteogenic differentiation on soft hydrogels', *Biomaterials*, Volume 268, 2021, 120543, ISSN 0142-9612, <https://doi.org/10.1016/j.biomaterials.2020.120543>.)

³Matrix mechanotransduction mediated by thrombospondin-1/integrin/YAP in the vascular remodeling (Yoshito Yamashiro, Bui Quoc Thang, Karina Ramirez, Seung Jae Shin, Tomohiro Kohata, Shigeaki Ohata, Tram Anh Vu Nguyen, Sumio Ohtsuki, Kazuaki Nagayama, and Hiromi Yanagisawa, 'Matrix mechanotransduction mediated by thrombospondin-1/integrin/YAP in the vascular remodeling, April 22, 2020, *Proceedings of the National Academy of Sciences of the*

United States of America, National Academy of Sciences, 117 (18) 9896-9905, <https://doi.org/10.1073/pnas.1919702117>)

⁴Loss of RAP2A Aggravates Cartilage Degradation in TMJOA via YAP Signaling (Qi H, Zhang Y, Xu L, et al. Loss of RAP2A Aggravates Cartilage Degradation in TMJOA via YAP Signaling. *Journal of Dental Research*. 2023;102(3):302-312. doi:[10.1177/00220345221132213](https://doi.org/10.1177/00220345221132213)) ⁵A spatial model of YAP/TAZ signaling reveals how stiffness, dimensionality, and shape contribute to emergent outcomes

(Kiersten Elizabeth Scott, Stephanie I. Fraley, and Padmini Rangamani, 'A spatial model of YAP/TAZ signaling reveals how stiffness, dimensionality, and shape contribute to emergent outcomes', May 14 2021, *Proceedings of the National Academy of Sciences*, National Academy of Sciences,

118 (20) e2021571118, <https://doi.org/10.1073/pnas.2021571118>)

¹"RAP2 Mediates Mechano-responses of Hippo Pathway - PMC - NCBI." 22 Feb. 2019, <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6128698/>. Accessed 23 Feb. 2023.

²"Controllable ligand spacing stimulates cellular mechanotransduction and" <https://www.sciencedirect.com/science/article/pii/S0142961220307894>. Accessed 23 Feb. 2023.

³"Matrix mechanotransduction mediated by thrombospondin-1/integrin" 22 Apr. 2020, <https://www.pnas.org/doi/10.1073/pnas.1919702117>. Accessed 23 Feb. 2023.

⁴"Loss of RAP2A Aggravates Cartilage Degradation in TMJOA via" 10 Nov. 2022, <https://pubmed.ncbi.nlm.nih.gov/36366779/>. Accessed 23 Feb. 2023.

⁵"A spatial model of YAP/TAZ signaling reveals how ..." 14 May. 2021, <https://www.pnas.org/doi/10.1073/pnas.2021571118>. Accessed 23 Feb. 2023.