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Extracellular Matrix Elasticity Gives Integrin a Sweet Change via a p53/miRNA-532/atp2c1 Axis

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Abstract: Extracellular matrix (ECM) elasticity affects the function of a variety of cells. Integrins are transmembrane receptors that considered to be a sensor of cellular mechanical stimulation. The activity of integrins is strongly influenced by glycans through glycosylation events and the establishment of glycan-mediated interactions. Our study found that the level of β1 integrin N-linked glycosylation was significantly down-regulated on softer ECM. Further, sialic acid is a common monosaccharide modified at the end of the sugar chain during N-glycosylation. We subjected the enriched sialylated glycoproteins to gel-based proteomic identification by tandem mass spectrometry and found that the chondrocytes seeded on stiff ECM have a higher glycosylation dynamics than soft ones.

Glycosylation of proteins is one of the important post-translational modification processes, which plays a key role in the correct folding, transport, localization and enzymatic hydrolysis of proteins. Glycosylation includes N-linked glycosylation and O-linked glycosylation, both of which are completed in the Golgi. Since glycosylation is common in proteins such as membrane proteins, secreted proteins, and transcription factors, the glycosylation process of proteins has a wide range of important effects on the migration, cycle, and differentiation of various cells, and with various diseases. Given the important effects of protein glycosylation, we believe that changes in the level of integrin glycosylation and the level of protein sialylation in cells may be one of the reasons for the wide-ranging effects of ECM elasticity on a variety of cellular functions.

By gene chip analysis and RT-PCR, Western Blotting, we found that softer ECM inhibited the expression of Calcium-transporting ATPase type 2C member 1 (SPCA1). SPCA1 is a calcium and manganese ion transporter on the Golgi apparatus (GA), and its expression level has a profound influence on the calcium ion and manganese ion concentrations in the GA. The concentration of calcium ions and manganese ions in the GA affects the activity of glycosylation enzymes, thereby affecting the level of protein glycosylation in whole cells. Therefore, we next quantified the Ca^{2+} concentration within GA using fluorescence energy resonance transfer (Fret)-based calcium biosensor. A calcium ion FRET probe containing a Golgi resident peptide end was transfected into cells, and we observed that the frequency of spontaneous calcium oscillations within GA were up regulated on stiff ECM cells. We thus suggest that ATP2C1 is involved in regulating glycosylation in response to ECM stiffness through Ca^{2+}.

We next explore the function and possible regulator of SPCA1. We found that ECM elasticity changed Integrin glycosylation via an miRNA-532/atp2c1 axis. miR-532 repressed atp2c1 and regulated SPCA1 expression through a specific 3′-UTR binding site. As detected by luciferase assays, p53 is in the upstream of a miR-532/atp2c1 axis. The accumulated nucleus p53 on soft substrate bound to the promoter of miRNA-532 to decrease atp2c1 expression and protein glycosylation. In conclusion, we indicated that a p53/miRNA-532/atp2c1 axis plays a pivot role in dynamic changes of Integrin β1 glycosylation. This revealed the mechanism, by which mechanical forces modulate Integrin glycosylation.

Keywords: ECM elasticity; glycosylation; Integrin; miRNA-532; SPCA1