STIFFNESS OF 3D COLLAGEN MATRICES REGULATES CDC42 ACTIVITY OF ENDOTHELIAL COLONY FORMING CELLS DURING EARLY VACUOLE FORMATION

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Recent preclinical reports have provided evidence that endothelial colony forming cells (ECFCs), a subset of endothelial progenitor cells, significantly improve vessel formation, largely due to their robust vasculogenic potential. While it has been known that the Rho family GTPase Cdc42 is involved in this ECFC-driven vessel formation process, the effect of extracellular matrix (ECM) stiffness on its activity during vessel formation is largely unknown. Using a fluorescence resonance energy transfer (FRET)-based Cdc42 biosensor, we examined the spatio-temporal activity of Cdc42 of ECFCs in three-dimensional (3D) collagen matrices with varying stiffness. The result revealed that ECFCs exhibited an increase in Cdc42 activity in a soft (150 Pa) matrix, while they were much less responsive in a stiff (1000 Pa) matrix. In both soft and stiff matrices, Cdc42 was highly activated near vacuoles; however, its activity is higher in a soft matrix than that in a stiff matrix. The observed Cdc42 activity was closely associated with vacuole area. Soft matrices induced higher Cdc42 activity, faster vacuole formation, and larger vacuole area than stiff matrices. Time courses of Cdc42 activity and vacuole formation data revealed that Cdc42 activity proceeds vacuole formation. Collectively, these results suggest that matrix stiffness is critical in regulating Cdc42 activity in ECFCs and its activation is an important step in early vacuole formation.

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