Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Investigating the role of mechanochemistry and ionic microenvironment in hydrogel-induced myoblast differentiation and calcium-driven glioblastoma stemness
（ハイドロゲル誘導性筋芽細胞分化とカルシウム駆動性神経膠芽腫幹細胞性におけるメカノケミストリーとイオン性微小環境の役割に関する研究）

We developed two functionalized hydrogel systems to reveal the molecular mechanism of the regulation of cell fate by the physicochemical properties of the microenvironment. The first study focused on the effect of the charge properties of the tumor microenvironment on the malignant phenotype of glioma stem cells (GSCs), and the second study explored the regulatory effect of the topological-chemical coupled microenvironment on myoblast differentiation. The study found that microenvironmental signals activated conserved transcriptional networks through a mechanical-biological coupling mechanism, providing new strategies for tumor treatment and tissue engineering.

1.Glioblastoma has a poor prognosis and a markedly low five-year survival rate. Glioma stem cells (GSCs) are regarded as a critical factor in recurrence, but the molecular mechanisms of GSCs generation in tumor microenvironment remain unclear. It has been reported that glioblastoma tissue is rich in positive charges due to the influence of the extracellular matrix and hypoxia. In this study, we developed adjustable, fully synthetic hydrogels to elucidate the effect of different charges on stemness induction. By varying the concentration of polymer-associated ionizable monomers, we synthesized hydrogels with surface charges ranging from negative to positive, and found that cationic hydrogels induced stem cell characteristics to the greatest extent. RNA sequencing revealed that cationic hydrogels enhance the expression of genes related to epithelial-to-mesenchymal transition, improve cell tolerance to temozolomide, and increase the expression of calcium channel proteins. Mechanistically, chromatin immunoprecipitation assays demonstrated that the cationic hydrogel activates the calcium ion-NFAT pathway, allowing NFATc1 and NFATc2 to translocate into the nucleus and act as transcription factors, thereby inducing c-Myc expression to promote glioblastoma stemness. The knockdown of NFATc1 and c-Myc significantly reduced cationic hydrogel-induced stemness, indicating that NFAT and c-Myc are crucial for this process. Spatial transcriptome analysis demonstrated that c-Myc is expressed surrounding palisading necrosis areas in addition to perivascular regions in human glioblastoma tissues. These results suggest that positively charged tumor microenvironment promote malignant phenotypes through calcium-NFAT-c-Myc-driven glioma reprogramming, providing a new strategy for glioma treatment.

2.Micropatterned surface substrates containing topographic cues offer the possibility of programming tissue organization as a cell template by guiding cell alignment, adhesion, and function. In this study, we developed and used a force stamp method to grow aligned micropatterns with tunable chemical properties and elasticity on the surface of hydrogels based on a force-triggered polymerisation mechanism of double-network hydrogels to elucidate the underlying mechanisms by which cells sense and respond to their mechanical and chemical microenvironments. In this work, we describe the impact of aligned micropatterns on the combined effects of microstructural chemistry and mechanics on the selective adhesion, directed migration, and differentiation of myoblasts. Our investigations revealed that topographically-engineered substrates with hydrophobic and elevated surface roughness significantly enhanced myoblast adhesion kinetics. Concurrently, spatially ordered architectures facilitated cytoskeletal reorganization in myocytes, establishing biomechanically favorable niches for syncytial myotube development through extracellular matrix (ECM) physical guidance. Reverse transcription PCR analysis and immunofluorescence revealed that the expression of differentiation-specific genes, myosin heavy chain, and myogenic regulatory factors Myf5 and

MyoD were upregulated in muscle cells on the aligned patterned scaffolds. These results suggest that the aligned micropatterns can promote muscle cell differentiation, making them potential scaffolds for enhancing skeletal differentiation.

The two studies jointly proved that: ① The charge/topological properties of hydrogels can be used as independent variables to analyze the biological effects of the microenvironment; ② The calcium signal-NFAT axis and ECM mechanical guidance affect cell fate through epigenetic remodeling and cytoskeletal reorganization respectively; ③ Material engineering strategies can accurately simulate pathological/physiological microenvironments, providing new ideas for targeted intervention. This study lays a theoretical foundation for the development of charge-responsive anticancer scaffolds and topological optimization tissue engineering platforms. In the future, the temporal regulation mechanism of the microenvironment can be further explored through spatiotemporal dynamic hydrogel systems.