学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称　博士(ソフトマター科学)　氏　名　孫　雁鵬

学 位 論 文 題 名

Study on the effect of stiffness of tumor microenvironment on cancer stem cell properties using hydrogels
(ハイドロゲルを用いた腫瘍微小環境の硬さががん幹細胞の特性に及ぼす影響に関する研究)

Cancer recurrence, therapy resistance, and metastasis remain significant challenges in oncology, primarily driven by cancer stem cells (CSCs), a subpopulation with self-renewal and tumor-initiating abilities. Recently, the mechanical properties of the tumor microenvironment (TME), particularly stiffness, have been recognized as key regulators of CSC behavior. Double-network (DN) hydrogels have been shown to induce cancer cells into CSC-like states, a phenomenon termed hydrogel reprogramming of adherent phenotype (HRAP). While this discovery highlights the significance of mechanical stimulation, the precise mechanisms by which TME stiffness regulates CSC properties remain unclear. This study addresses this gap by investigating how varying hydrogel stiffness influences CSC induction and maintenance.

The first part of this research focused on H-Ras- and Src-transformed NIH3T3 cells cultured on PAMPS hydrogels with varying stiffness levels. H-Ras-transformed cells exhibited significant upregulation of CSC markers, including *Sox2*, *Nanog*, and *Oct4*, when cultured on hydrogels with a stiffness of approximately 10 kPa, resembling tumor tissue rigidity. In contrast, Src-transformed cells failed to exhibit similar induction, indicating that oncogene-specific signaling is essential for stiffness-dependent CSC generation. Mechanistic investigations identified mechanosensitive calcium channels, particularly members of the TRPC and TRPV families, as key mediators of calcium influx in response to soft environments, leading to activation of the ERK signaling pathway. Desmoplakin, a membrane-associated protein, was also identified as a critical component interacting with 10 kPa hydrogels, contributing to CSC induction.

In the second part of the study, the heterogeneity of CSC responses to stiffness was examined across multiple cancer cell lines, including models of brain, bladder, pancreatic, cervical, synovial sarcoma, and osteosarcoma. The results revealed diverse stiffness preferences among cell lines. Some, such as MIA PaCa-2, favored soft environments (~10 kPa), whereas others, like KMG4 and UM-UC-3, exhibited enhanced CSC marker expression under stiffer conditions (~200 kPa). A key finding was the identification of the leptin receptor (LEPR) as a major mediator of stiffness-induced CSC properties in stiff environments. LEPR expression increased on stiff hydrogels, and functional studies showed that its knockdown or inhibition significantly reduced CSC marker expression. Mechanistically, LEPR activated the ERK-RSK-YB1 signaling pathway, linking mechanical stiffness to CSC maintenance.

This study demonstrates that TME stiffness regulates CSC behavior through distinct molecular mechanisms depending on the mechanical environment. In softer environments,

Desmoplakin and TRP calcium channels facilitate stiffness sensing, while stiffer environments promote LEPR-mediated CSC maintenance via ERK-RSK-YB1 signaling. These findings underscore the heterogeneity of CSC responses to mechanical cues and suggest that targeting mechanosensitive pathways may provide novel therapeutic opportunities to disrupt CSC properties and combat therapy resistance.By using stiffness-mimicking hydrogels as a platform, this research offers valuable insights into the mechanobiology of CSC regulation. It lays the groundwork for developing innovative therapies aimed at modifying the mechanical aspects of the TME to suppress CSC properties and improve cancer treatment outcomes.