学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Study on nuclear localization of myosin regulatory light chain in response to substrate stiffness
（基質の硬さに依存したミオシン調節軽鎖の核局在についての研究）

Cells react to physical factors like substrate stiffness, yet the mechanisms are not fully understood. Recent studies have highlighted that increased substrate stiffness enhances the di-phosphorylation of myosin regulatory light chain (MRLC). Furthermore, it's been observed that this di-phosphorylated form of MRLC (2P-MRLC) is involved in controlling gene expression within the cell nucleus. This suggests that MRLC might play a key role in how cells respond to stiffness by activating specific genes. In our research, we focused on uncovering how MRLC influences cellular reactions to stiffness. Previous findings have indicated that 2P-MRLC is present both in the nucleus and the cytoplasm. Our objective was to determine if substrate stiffness affects the distribution of 2P-MRLC, particularly its presence in the nucleus. To this end, we used soft and stiff polyacrylamide gel substrates with stiffness levels of 0.4 kPa and 271 kPa, respectively. We cultured human cervical cancer cells (HeLa cells) on these substrates and examined the localization of MRLC. Our observations revealed a distinct pattern: on stiff substrates, 2P-MRLC predominantly accumulated in the nucleus of HeLa cells, while on softer substrates, it remained in the cytoplasm. Furthermore, disruption of the actin cytoskeleton, which is important for the transduction of substrate stiffness into the cell, suppressed the nuclear localization of 2P-MRLC. In addition, we focused on ZIPK, which functions to di-phosphorylate MRLC, and examined whether ZIPK regulates the nuclear localization of 2P-MRLC. We found that both inhibition of ZIPK and suppression of its expression in HeLa cells on a stiff substrate suppressed the nuclear localization of 2P-MRLC. We also found that ZIPK translocate to the nucleus in response to substrate stiffness via the actin backbone. From these results, These results reveal that substrate stiffness, via the actin cytoskeleton, induces nuclear localization of 2P-MRLC, and this localization is regulated by ZIPK. Then, we aimed to specific genes that are influenced by the nuclear presence of 2P-MRLC in response to substrate stiffness. Through qPCR screening, we investigated gene expression changes linked to both stiff substrates and MRLC. This led to the finding that the expression of MafB, a gene known to induce apoptosis during limb development, is reduced under conditions of stiff substrate and increased MRLC expression. Building on this, we hypothesized that the decreased MafB expression, triggered by the nuclear accumulation of 2P-MRLC in response to stiff substrates, might lead to reduced apoptosis. To test this hypothesis, we assessed the levels of cleaved-caspase3, an indicator of apoptosis, in various cell groups: control cells, cells with reduced MRLC (MRLC-KD), and cells with both MRLC and MafB reduced (double KD). Our results showed that reducing MRLC alone increased apoptosis, as indicated by higher cleaved-caspase3 levels. However, simultaneously reducing MafB reversed this increase in apoptosis in the MRLC-KD cells. In conclusion, our research provides novel insights into cellular respond to stiffness, particularly highlighting the role of 2P-MRLC's nuclear localization in modulating apoptosis through the suppression of MafB expression. This understanding could be crucial in deciphering how cells respond to varying mechanical environments, particularly in the context of stiffness-related cellular processes.