論 文 の 内 容 の 要 旨

論文　題目

Lattice Micropatterns for Optimizing Osteogenic Differentiation of Murine Mesenchymal Stem Cells

(マウス間葉系幹細胞の骨分化を最適化する格子微細パターン)

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Unraveling the underlying mechanism and key factors in controlling the differentiation of stem cell is one of the major areas in stem cell research. Coaxing stem cells into specific cell lineages with high robustness is an indispensible requisite in various applications of them for the efficient clinical uses. Although recently a growing number of reports demonstrate that topography or geometry of the substrate also plays an important role in the fate of the stem cells, most of these studies are usually conducted by a few distinct patterns such as simple lines, posts or other specified shapes. As a result, there is a lack of quantitative and comprehensive analysis of the relationship between topographical variation and the differentiation of stem cells. In this study, I systematically examined the effectiveness of topography variation on osteogenic differentiation with several micropatterned substrates after designing a series of lattice micropatterns. For this study, I used seven kinds of micropatterns ranging from flat to 8 μm (flat, 1, 2, 3, 4, 6, 8 μm) in the interval length, with 1 μm height in all micropatterns. I examined the expressions of the osteogenic markers, alkaline phosphatase, collagen type-I and osteocalcin in murine mesenchymal stem cells cultured on the prepared lattice micropatterns. The results show that the effectiveness of the osteogenic differentiation has a peak value on the 3 μm interval length. This demonstrates that the differentiation of stem cells can be controlled by not only chemical factors but also topographical factors of substrates. Additionally, I empirically observed that the focal adhesions maturation were highly dependent on the topography of the substrate although the shapes, morphology and spreading of cells on different substrates do not have meaningful difference. Based on this empirical observation, I furthered my study for in-depth understanding the underlying pathway of topographical cue on the stem cell differentiation by first examining the focal adhesion and actin cytoskeleton property. On this perspective, first I compared the formation of focal adhesions of cells cultured on the different

substrates; flat one (without topography) and micropattern (2 μm lattice pattern with 3 μm interval length and 1 μm depth). I demonstrate that not only the focal adhesion, actin polymerization is also strongly developed at the cells cultured on the micropattern. These different cell behaviors let me focus the RhoA protein, one of small GTPase family and subsequent pathway since a couple of reports have demonstrated RhoA-activated cells have the enhanced focal adhesions and actin activity (polymerization) at the same time. Based on the cell behaviors regarding focal adhesion and actin activation and related previous literatures, I made a hypothesis about the cellular steps on the topography-mediated focal adhesion formation and actin activation as follows: 1) initial cell adhesion and topography sensing step via integrins since previous studies reported integrins, especially β1, act as a mechanosensor to probe the surrounding, 2) cell spreading, 3) topography-dependent activation of RhoA-ROCK pathway and 4) myosinII stimulation 5) actin activation (substrate contraction) through myosin II and guided and enhanced focal adhesion formation. By inhibiting the Rho-related kinase (ROCK) and subsequent myosin light chain kinase (MLCK), I found that the focal adhesion formation is highly decreased by those inhibitions. Not only so, the topography-dependency of focal adhesion formation is highly decreased. These suggest that RhoA pathway is critical for the formation of the focal adhesion. Moreover, at the cells on the micropatterns, the integrin α_5 is highly expressed and focal adhesion kinase is also more activated (pY397). This result demonstrates that activation of integrin α_5 and phosphorylation of FAK are accompanied with the maturation of focal adhesion and myosin II-generated cytoskeletal contraction. Through my studies (topographical effect on stem cell differentiation and then focal adhesion maturation including actin cytoskeleton activation), I could speculate the topographical effect on the stem cell function (esp., differentiation) as follows. First, the topography-mediated enhancement of focal adhesion and actin cytoskeleton polymerization may strongly influence the differentiation of the stem cells. The rational behind this speculation is that a couple of previous reports have also shown that RhoA activation (by chemical and physical cues) enhances the specific differentiation efficiency of stem cells. Therefore I could rationalize that the appropriately well-designed topography of the substrate upregulate the activation of RhoA pathway which then results in the subsequent enhancement of focal adhesion, actin polymerization and the differentiation of stem cells.