

주기적 인장력 제공 및 파이브로넥틴 마이크로 패턴닝을 이용한 세포의 방향성 및 분열조절 연구

이은경*, 이승재**, 김 천호***, 손영숙*

Control of Cell Alignment and Cell Proliferation by Cyclic Strain and Fibronectin

Physical stimuli such as tension and compression are known to play an important role on the regulation of cell function such as proliferation, apoptosis, and cell survival. The intracellular signaling pathway from physical stimuli seem to share with that of growth factors and extracellular matrix. In this study, we showed that cyclic strain stimulated cell survival and cell proliferation under serum-free condition and further explored early signaling pathway leading to the stimulation of cell survival and cell proliferation under these condition respectively. When equibiaxial cyclic strains (20 % maximal strain, 0.5 Hz frequency) using FlexCell 4000T tension system was applied to dermal fibroblasts under the serum free condition on flexible rubber plate, the apoptosis of dermal fibroblast mediated by serum deprivation was decreased by 5 folds and stimulated cell proliferation by 2 folds. For the identification of early signaling leading to the cell survival, the dermal fibroblasts applied by the 20 % equibiaxial strain for 10 min, 30 min, 1 hr, and 24 hr were harvested and activation profiles of Erk1/2, AKT and JNK1/2 were examined by western blot analysis. Cyclic strain-stimulated activation of Erk1/2, AKT and JNK1/2 was all detected at as early as 10min post strain and disappeared at 30 min post strain. At serum free

condition, p21 was not detected but detected at 10 min post strain and gradually increased up to 1 hr post strain. p21 was again completely disappeared at 24 hr post strain. Cyclic strains also stimulated expression of fibronectin but didn't affect the expression of MMP. From our data, we concluded that cyclic strain stimulate cell survival of serum deprived fibroblasts probably through the genuine cell survival signaling pathway.

Cell adhesion is an important step for cell shape, cell survival, and proliferation for anchorage-dependent cells, whose dimension can be controlled by micro-or nano patterning of cell adhesive extracellular matrix. We made the replica molding into silicon via photomask in quartz by E-beam lithography, and fabricated polydimethylsiloxane (PDMS) stamp using the designed silicon mold. Fibronectin of 2 μ m width strip was spaced from 2 μ m to 20 μ m and its micropatterning was confirmed by immuno- fluorescence staining. We tested whether fibroblasts can attach only on the fibronectin micropatterned surface. Fibroblasts were attached only on the fibronectin-coated micropattern at 4 h after plating but their strict cell alignments were disturbed on 2 days culture, which is probably due to endogenous fibronectin secretion. The cell alignment at the gap smaller than 10 μ m was random but that at the gap larger than 10 μ m was longitudinal along the fibronectin micropatterns covered by two or three strips. This discrepancy in cell alignment may be reflected by filopodia protrusion, which may be critically limited by the adhesion-gap size over 10 μ m. Longitudinally

*경희대학교 생명과학대학/근골격계바이오장기센터

**엠씨티티 연구소

***원자력의학원 생체조직재생연구실

oriented fibroblasts on the fibronectin-patterned surface were lower in cell proliferation based on BrdU labeling. Therefore our fibronectin micropatterning suggests that filopodia protrusion may be limited by the adhesive gap larger than 10 μ m and the confinement of cell dimension on the longitudinal and narrow surface also negatively affect cell proliferation even though sufficient surface area are provided longitudinally.

후 기

이 연구는 손영숙 박사에게 지원된 보건복지부 근골격계 바이오 장기센터와 원자력 중장기 사업에 의해 수행되었음.