

Polarity control at microgrooved structures in migrating fibroblast

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Abstract

Cell migration is driven by the protrusion at the leading edge, adhesion formation, generation of traction stress, and retraction and detachment of the rear edge. These steps are remarkably affected by the micro-/nano-structure of the extracellular matrix underlying the migrating cell [1]. In previous studies, we have systematically analyzed the transient changes in migratory behaviors of epidermal cells encountering microgrooved structures, and have clarified cell migratory characteristics depending on the groove width and its arrangement [2][3].

In this study, we systematically analyzed the migratory behavior of fibroblasts which are the major mesenchymal cell type in connective tissue. We fabricated cell culture substrate consisting of microgrooves with various sizes and cultured fibroblasts on the substrate. Then we investigated the migratory behavior at the boundary with a single line groove.

The fibroblast migratory behavior at the single line groove was highly depending on the groove width. The fibroblasts crossed over the narrow single line groove with 1.5 μm in width and 20 μm in depth, whereas turned at a wider groove with 4 - 10 μm in width and 20 μm in depth. The fibroblast migratory behavior was different from that of the epidermal cells. We hypothesized that the difference in the spatial distribution of the cell-substrate adhesions would be key factors in determining the migratory behavior at the grooved structure. To test this hypothesis, next we quantitatively analyzed the distribution of focal adhesions in Swiss3T3 fibroblasts. The quantitative analysis demonstrated that the distance between the neighboring focal adhesions near the leading edge was longer than 1.5 μm . These results indicate that fibroblast migratory behavior at a single line groove could be determined based on whether or not neighboring focal adhesions can form over the groove. Consistent with the idea, the focal adhesion perturbed fibroblasts exhibited migratory behavior less depending on the groove width. Specifically, the perturbation of the local distribution of focal adhesions demonstrated that disappearance of focal adhesions near the leading edge led to a defect of cell turning behavior at a wider single line groove. Taken together with the results of the fibroblasts migration assay, the focal adhesions near the leading edge are assumed to contribute for the polarity change at a wider grooved structure.

Further studies to characterize the dynamics of actin cytoskeleton and focal adhesions by a live cell fluorescence microscopy and other perturbation methods will provide a more clear picture of the mechanism of polarity control in cells in response to micro-topography.

[1] Miyoshi and Adachi (2014) Tissue Engineering Part B, vol. 20, pp. 609-627

[2] Miyoshi et al. (2012) Biomaterials, vol. 33, pp. 395-401

[3] Miyoshi et al. (2010) Biomaterials, vol. 31, pp. 8539-8545

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