A role of F-actin and RhoA-Rock signaling in nanotopography-induced cell regulation

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Statement of Purpose

Nanotopography can influence cell behavior ranging from cell proliferation and migration to differentiation (*1*). However, the underlying mechanism of topography-induced cell behavior has scarcely been investigated. How does surface feature in nano-scale significantly change the cell behavior and cell fate?

Small Rho GTPases regulate various cell functions including migration, polarization and gene expression by interacting with effector molecules that initiate various signaling cascades. We have previously demonstrated that the morphology of smooth muscle cells and human mesenchymal stem cells (hMSCs) is significantly influenced by nanogratings, showing extensive alignment and elongation of both cytoskeleton and nuclei (*2*). We hypothesize that this phenomenon is related to the rearrangement of cytoskeleton and integrin clustering that regulates signal transduction pathways, such as the RhoA/Rock pathway.

Methods

Micro- and nano-scaled patterns on tissue-culture polystyrene (TCPS) were fabricated by nanoimprint lithography (NIL). The patterns were also replicated on poly(dimethylsiloxane) (PDMS) by soft lithography. Human MSCs (Cambrex) were cultured in MSCGM proliferation medium (Cambrex) on different patterns at a density of 2000-4000 cells/cm^2. Pharmacologic inhibitors, cytochalasin D, C3 exoenzyme, Y27632 were used to block F-actin arrangement, Rho, Rho associated kinase, respectively. The cytoskeleton, cell-substrate interaction and structure of focal adhesion were studied by fluorescent and immuno-fluorescent staining. RNA was isolated from the cells cultured on the patterns at day 7 for RT-PCR and real-time PCR analyses. Expression of integrin, vinculin, and FAK was determined by immunoblot analysis. Changes in cytoskeleton arrangement, protein expression and differentiation were examined in the presence of the inhibitors.

Results and Discussion

In the elongated hMSCs cultured on nanopatterned TCPS and PDMS, the expression of RhoA, ROCK1, LIM kinase, MAPK1 and MAPK3 was down-regulated compared to unpatterned controls (Figure 1). Expression of Cdc42 was up-regulated in the hMSCs cultured on nanopatterned PDMS compared to the unpatterned PDMS control; however, the expression of Cdc42 was reduced compared to TCPS samples.

The distribution and area of focal adhesion were significantly altered in the hMSCs cultured on nanopatterns compared to unpatterned samples. The area of focal adhesion was reduced and the focal adhesion complex (FAK) was mainly localized at the poles of the elongated hMSCs (Figure 2). Expression of vinculin was also reduced in *h*MSC cultured on nano-gratings of both TCPS and PDMS.

We have previously reported that neuronal gene markers were upregulated in hMSCs cultured on nano-gratings (ref). Upon addition of cytochalasin D, not only the F-actin cytoskeleton was disrupted, the up-regulation of neuronal gene markers was also diminished. Moreover, the disruption of F-actin cytoskeleton also reversed the down-regulation of MAPK1, MAPK3 and ROCK1 observed in the hMSC cultured on nanopatterns; however, disruption of F-actin did not affect the expression level of the kinases in cells cultured on non-patterned surface. The result suggested the involvement of F-actin in nanotopography-induced regulation in the RhoA/Rock pathway.

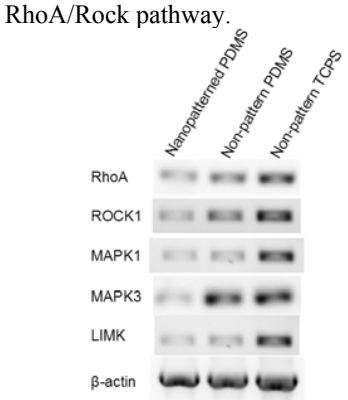

Figure 1. Downregulation of RhoA, ROCK1 and MAP kinases in hMSC cultured on nanopattern.

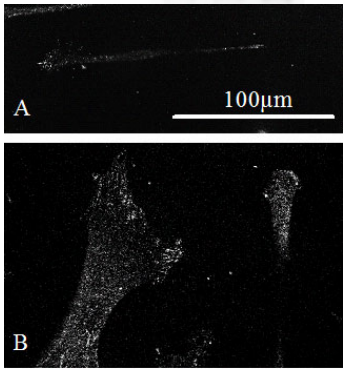

Figure 2. Expression of FAK ([Tyr397] Phospho-specific) in *h*MSCs cultured on (A) nanopatterned PDMS and (B) unpatterned PDMS.

Conclusions:

When hMSCs cultured on nanopatterned PDMS and TCPS, not only were the cytoskeleton and the focal adhesion organization significantly changed, the expression of Rho/Rock was also considerably regulated. As the Rho/Rock signaling transduction pathways are closely associated with cytoskeleton arrangement, gene transcription and cell cycle regulations, the Rho GTPases are likely to play an important regulatory role in the nanotopography-induced cell behavior.

References:

1. Yim EK *et al.*, Nanomedicine: Nanotechnology, Biology, and Medicine. 2005: 1: 10-21.
2. Yim EK *et al.*, Biomaterials. 2005: 26: 5405-5413.