Response of Human Embryonic and Adult Mesenchymal Stem Cells to Nanotopography

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Introduction

Cell behaviour and cell fate are influenced by biomolecular and topographical cues in the natural microenvironment. Topographical cue can influence cellular responses from attachment to differentiation and tissue production. In a recent study, we have shown that the cell behaviour of bovine pulmonary artery smooth muscle cells (SMC) is significantly different when cultured on surface with nano-gratings compared to monolayer culture on tissue-culture polystyrene (TCPS) surface [1].

Hypothesizing that nano-topographical cues would have even a stronger effect on more primitive cells, we examined the differentiation of human embryonic stem cells and human mesenchymal stem cells on poly(dimethlsiloxane) (PDMS) surfaces, patterned with gratings of 350 nm width and 350 nm depth. Both cells showed aligned and elongated morphology as well as increased expression of neuronal lineage and muscle lineage markers. This study demonstrated the significance of nanotopography in directing differentiation of embryonic and adult stem cells.

Materials and Methods

The nanoimprinted PMMA-coated Si was used as a master sample for the replica molding [1, 2]. After the fluorinated master was washed with 0.01% Triton X, degassed PDMS solution with cross-linking reagent (Sylgard 184 Silicone Elastomer Kit, Dow Corning) was poured onto the fluorinated wafer and baked at 50 °C overnight.

Human embryonic stem (hES) cells (H1) were obtained from WiCell and human mesenchymal stem cells (MSC) were obtained from Cambrex. The cells were cultured in the proliferating media specified by their suppliers. Human ES cells and hMSC were seeded at 1000 cells/ cm^2 and 4000 cells/ cm^2 on the nanopatterned PDMS samples, respectively.

Retinoic acid (1μM) was added to the ES medium, in which the bFGF was withdrawn. A higher concentration (30μM) was also studied in hMSC culture. Cell morphology was examined with F-actin cytoskeleton staining. Proliferation was examined with BrdU staining. Neuronal-stem cells marker GFAP, Tuj1, MAP2, nestin and synaptophysin were examined with immunofluorescence staining. Gene expression was analysed with RT-PCR (Qiagen one-step RT-PCR kit), microarray analysis (Superarray) and quantitative real-time PCR.

Results and Discussion

When hMSCs were cultured on the nanopatterns in their proliferation media, the hMSCs, as well as their nuclei, were aligned and elongated along the direction of the gratings (Figure 1). Similarly, hES cells adopted different types of morphology, including elongation along the grating axis or remained as aggregates.

Gene profiling with RT-PCR showed an upregulation of neuronal markers, such as MAP2 and tyrosine hydroxylase, muscle markers and vascular marker in hES and hMSC during the period of culture on the nanopatterned PDMS compared to non-patterned controls (Figure 2). In the presence of retinoic acid, expression of neuronal markers was also observed in hES cultured on non-patterned PDMS. Addition of retinoic acid to the medium enhanced the expression of the neuronal markers, such as SOX2 and GFAP, in hMSC.

Quantitative analysis of MAP2 showed a significantly enhanced expression of MAP2 in the hMSC cultured on the naopatterned PDMS with or without RA, when compared to non-patterned PDMS or TCPS controls. Microarray analysis showed most of the genes related to neuronal differentiation and neuronal function were induced or regulated when hMSC were cultured on the nanopattern.

Mature neuronal markers such as Tuj1 were detected by immunostaining in hES and hMSC cultured on nanopatterned surfaces. Other neuronal markers such as MAP2 were also detected in hMSC cultured on nanopatterned samples.

Compared to hMSC cultured on gratings with width of 1μm or 10μm, hMSC cultured on the nanopattern showed significantly reduced proliferation and higher MAP2 expression, suggesting nano-patterns could have a more significant effect on cell proliferation and differentiation compared to micro-patterns.

Conclusion

The cell morphology and gene expression of human ES cells and MSC cultured on nano-gratings were significantly different from those on non-patterned and micro-patterned controls. The significance of nanotopography in directing adult stem cell differentiation was shown in this study. A combination of nano-topographical and biochemical cues might create a potent and synergistic condition for manipulating stem cells for tissue engineering and cell therapy applications.

Reference

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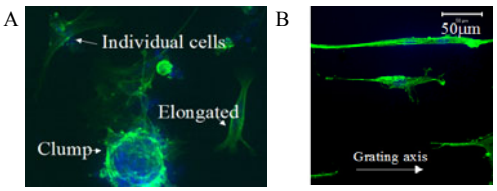

Figure 1. Confocal microscopy of hES (A) and hMSC (B) on nano-patterned PDMS.