Dynamic Force Generation by Neural Stem Cells

Peng Shi, Lance Kam
Columbia University, New york, NY 10027

Introduction

Neurogenesis is a central process in development and function of neural tissues. This involves an exquisite degree of coordination of cell behavior, balancing senescence, proliferation, migration, and differentiation of neural stem cells over a wide range of spatial and temporal scales. The mechanical response of the extracellular environment to cell-generated forces is a powerful modulator of cell function, including adhesion, spreading, migration, and differentiation, and may be an important cue in coordinating stem cell function. This study investigates the neural stem cell's mechanical interaction with the extracellular environment and its relation to cellular function by profiling force generation over the course of differentiation. Driven by the concept that cells apply forces to the environment to measure and respond to rigidity, we seek to identify specific cellular functions that are sensitive to this mechanical property.

Methods

We adopted the traction force microscopy technique introduced by Tan and colleagues [1] to map cellular forces. In this method, arrays of microscale, elastomer pillars are used as the cell culture substrate (Fig. 1A). The local, net force applied to each pillar can be estimated from this deflection, providing a simple, direct approach to mapping cellular forces. For this study, we used pillars (1 μm diameter, 7 μm height, 2 μm pitch) fabricated out of PDMS (E ~2MPa). Neural stem cells (NSC) derived from the subventricular zone (SVZ, a site of continued neurogenesis through adulthood) of P11 juvenile rats were cultured on the laminin coated micro-pillar arrays. After seeding, NSCs were cultured in expansion media (bFGF supplemented) for 1 day and then transferred to FGF-free differentiation media [2] containing retinoic acid and forskolin, as indicated in Fig. 1B. Deflection data for each pillar was extracted by using a multiple particle tracking program and converted to traction forces according to linear elastic theory.

Results

SVZ NSCs cultured on the pillar arrays was similar to that on standard cell culture surfaces, as compared by morphology and pattern of differentiation (determined through immuo-staining for lineage-specific markers, nestin, β III-tubulin and GFAP (Fig. 1C, D). Traction forces exerted by these cells (Fig. 1E, F) are highest, in the sub-nN range, during expansion (day 1 *in vitro*) as illustrated in Fig. 2. Following transfer to differentiation media, the net forces applied by the cells to the pillar arrays decreases with time in culture, as shown in Fig. 2. Data of micropillar deflection at 3 different time points (1, 4, 8 DIV) was significantly different from each other (Kruskal-Wallis methods, alpha < 0.05). By 7 days, only minor deflection could be identified; the timescale of this change in traction forces mirrored that of cell differentiation. As a control, we examined cells that were cultured on the pillar arrays for 8 days in expansion media, their forces were comparable to that of cells in culture for 1 day; which confirms that higher traction forces are associated with the undifferentiated phenotype, rather than an artifact of time in culture on these arrays.

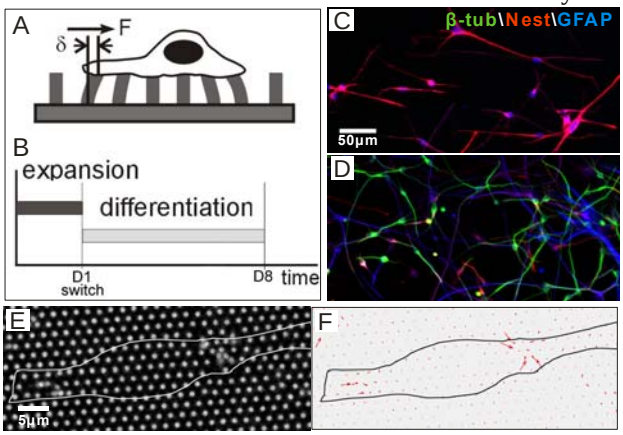

Figure 1. (A) Schematic of a NSC cultured on micro-pillar arrays. (B) Time frame of cell culture. (C) NSCs in expansion media (day 1) and after differentiation (day 8) stained for Nestin (red), β III-tubulin (green) and GFAP (blue). (E) Micropillars contracted by a cell 6 hours after seeding. (F) The correspondent force field mapped form the deflection of each pillars.

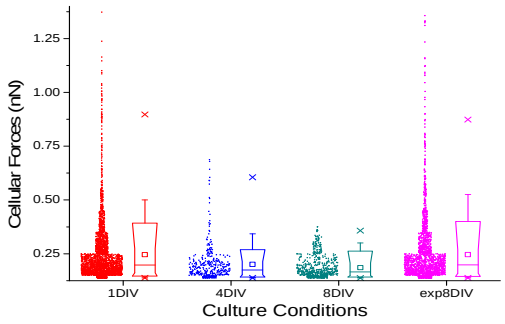

Figure 2. Boxplot of force strength measured at different stages throughout the course of differentiation (1, 4, 8 DIV), and data for control experiments, in which NSCs were cultured in expansion media for 8 days.

Conclusions

The forces exerted by neural stem cells onto an underlying cell culture surface are highest during expansion of this pool, and decrease over the course of differentiation. These results suggest that cellular functions associated with expansion and maintenance of the stem cell pool will be more sensitive to substrate rigidity than cells that have undergone differentiation. Future work will be focused on identifying components and mechanism that drive the changes of NSC's mechanical responses.

References

1. Tan JL. PNAS. 2003; 100(4):1484-9.
2. Palmer TD. J Neurosci. 1999; 19(19):8487-97.

Acknoledgement

This work is supported by NIH Roadmap for Medical Research (PN2 EY016586).