Nanofiber-Induced Wnt5a Modulates Cellular Reorganization of Dental Pulp Cells

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Statement of Purpose: Nanofibrous engineered matrices that mimic the morphology of natural collagen fiber have been shown to facilitate cellular behaviors. However, the underlying molecular events are poorly understood. Odontoblast is a long columnar, polarized cell responsible for dentin formation. In this study, we observed that the fibrous engineered matrix induced the differentiation of dental pulp cells to show the features similar to odontoblasts including expression of markers and morphological alteration. Also, it was demonstrated that nanofiber-induced Wnt5a mediated the cellular reorganization and expression of the molecules involving planar cell polarity (PCP).

Methods: Polystyrene nanofibrous matrix was fabricated from 12% (w/v) polystyrene solution in N,N-dimethylformamide by electro-spinning. Mouse or human dental pulp cells were seeded and cultured on polystyrene fiber matrix (PSF) and tissue culture dishes (TCD). It was checked the expressions by quantitative real-time PCR and by western blot analyses. Actin, mitochondria, nucleus, and cell membrane were stained and observed under confocal microscope. To check the involvement of Wnt5a on the alterations, the cultures were treated with recombinant human Wnt5a (rhWnt5a) or siRNA against Wnt5a. NFATc1 was examined as a component of Wnt5a downstream signaling by inhibition of translocation to nucleus, forced expression of NFATc1, or the knockdown.

Results: PSF induced higher expressions of Wnt5a, NFATc1, and PCP molecules (Vangl1, Scrb1), compared with the culture on TCD. The cells on PSF showed more elongated and polarized morphology. PSF-induced Wnt5a modulated these alterations, which was confirmed by treatments with rhWnt5a and si-Wnt5a RNA. Nuclear translocation of NFATc1 was increased in cells on PSF. rhWnt5a treatment increased the level and translocation of NFATc1, and these were abrogated by Wnt5a knockdown. In addition, we observed that overexpression of NFATc1 increased expressions of Wnt5a and PCP molecules, and the knockdown decreased them.

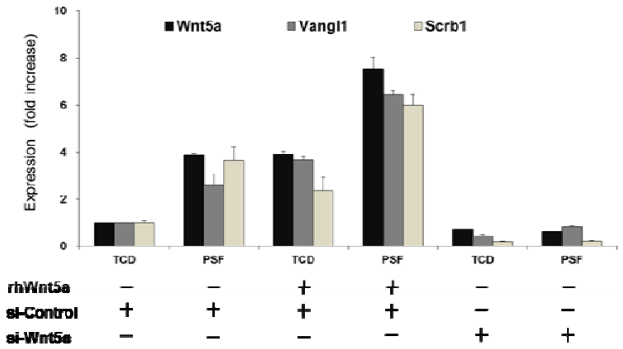

Figure 1. Effect of Wnt5a on expression of PCP molecules in dental pulp cells.

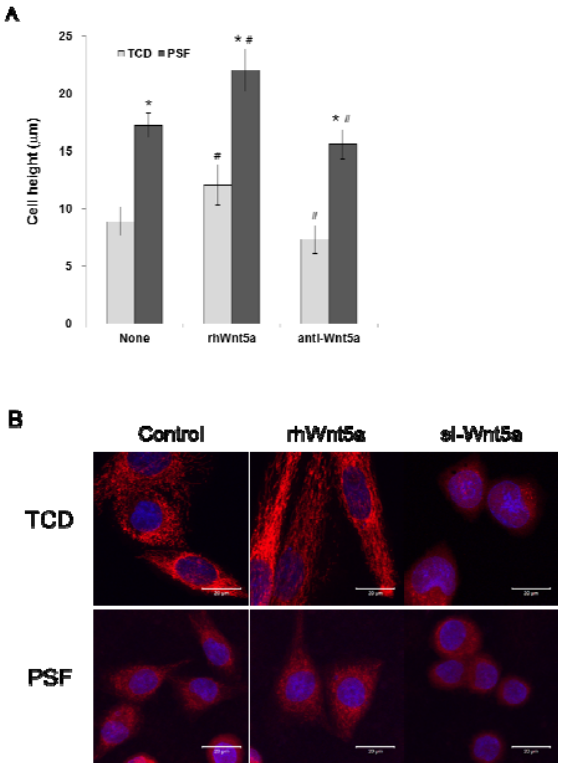

Figure-2. Morphology of Dental Pulp Cells on PSF and TCD. (A) Cell heights * Significant difference from TCD ($p<0.05$), # Significant difference from none treated group (B) Confocal microscopic images of mitochondria-staining with MitoTracker (Cell Signaling).

Conclusions: These results in this study provide evidence that fibrous engineered matrix induces the cellular reorganization of the dental pulp cells, which the nanofiber-induced Wnt5a mediates through NFATc1.