Supporting Information

Fibroblasts enhance migration of human lung cancer cells in a paper-based co-culture system

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Keywords: tumor cells, fibroblasts, migration, coculture

Materials and methods

Whatman lens paper, 4,6-diamidino-2-phenylindole (DAPI), and Draq5 were supplied by Sigma-Aldrich (St Louis, MO). Antibodies to TGF-beta and IgG were obtained from R&D Systems (Minneapolis, MN). Antibodies for E-Cadherin and vimentin were bought from Cell Signaling Technology (Danvers, MA). Trypsin-EDTA, penicillin-streptomycin, fetal bovine serum (FBS), GlutaMax, Dulbecco's minimal essential medium (DMEM) medium, and Dulbecco's phosphate buffered saline (DPBS) were purchased from Invitrogen (Carlsbad, CA). The 16% (v/v) paraformaldehyde solution was purchased from Electron Microscopy Sciences (Hatfield, PA). All reagents were used as received without further purification.

Preparation of paper scaffolds

A 40 μm-thick polyvinylchloride (PVC) sheet was laser cut to create the holes for cell-seeding zones. The PVC sheet was placed between two layers of lens paper and hot-embossed at 450 °F for 30 sec. The resulting PVC-infused paper scaffolds were cut and sterilized by autoclaving. The scaffolds were kept sterile at room temperature until further use.

Cell cultures

The tumor cells (VXN2) and fibroblasts (TF) were isolated from the biopsies of human patients. The VXN2 cells (Vertex Pharmaceuticals Inc.) were isolated *in vitro* from a primary non-small-cell lung carcinoma (NSCLC) tumor sample, which was first expanded in mice. The TF cells (Vertex Pharmaceuticals Inc.) were isolated directly from a primary NSCLC sample. Both cell types were cultured on tissue-culture treated plastic in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 1% (w/v) Glutamax, and 1% (v/v) penicillin/streptomycin. The cell cultures were maintained in a standard 37°C incubator equipped to provide 5% CO_2. We changed the medium every other day to provide a fresh environment for cells.

Co-cultures of human lung cancer cells and tumor fibroblasts in multi-layered paper stacks

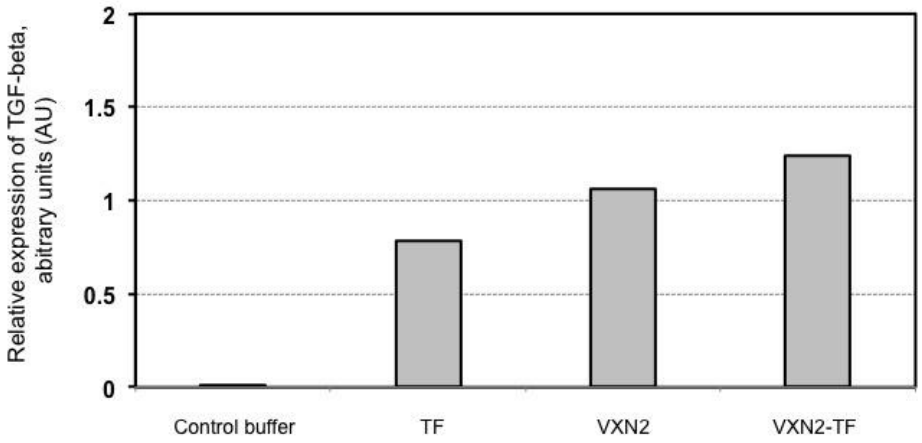
In the co-culture experiments, we used VXN2 (passage number 10-19) and TF (passage number 3-12) cells. After harvesting the cells from tissue cultures flasks, we labeled the VXN2 cells by cell tracker green. We seeded them in the culture zones of the double lens papers and assembled them in different configurations. After culturing the paper stacks for three days, we separated the layers from the scaffolds and scanned them with high resolution Typhoon scanner. We quantified the amount of fluorescence for each cell-seeded zone by using the NIH Image J software. Subsequently, we generated the bar plots based on the percent (%) of VXN2 cells that are present in each layer. We performed the experiments in triplicates for each condition.

Immunocytochemistry for Epithelial-to-Mesenchymal Transition (EMT)

To assess the epithelial and mesenchymal phenotype of the tumor cells, they were tested for expression of E-Cadherin and Vimentin. The samples were fixed in a 4% (v/v) paraformaldehyde solution and permeabilized using 0.5% (v/v) Triton X-100. A 10% (v/v) solution of goat serum was used to block the non-specific protein binding. The samples were incubated with 1/100 diluted primary antibody mixture for E-Cadherin and vimentin overnight at 4°C. 1/100 diluted secondary antibodies were then used to incubate the samples at room temperature for 1 h. The samples were washed with PBS and imaged using a Zeiss LSM710 confocal microscope (Carl Zeiss Microscopy, LLC, Thornwood, NY).

Statistical analysis

The experimental data was analyzed by GraphPad Prism (Version 4.02, La Jolla, CA). The statistical differences between groups were determined by one-way ANOVA tests with Bonferroni comparisons. p-values < 0.05 are considered statistically significant (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).



Supplementary Figure S1. ELISA assay (Life Technologies) for TGF-beta. TGF-beta was secreted by VXN2, TF, and co-cultured VXN2-TF cells. The absorbance values at 405 nm were normalized to the signal that was obtained from the media. As expected the signal for TGF-beta in the control experiment was undetectable.