Fibroblasts Enhance Migration of Human Lung Cancer Cells in a Paper-Based Coculture System

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The microenvironment of tumors includes extracellular matrix proteins such as collagen, fibronectin, and laminin, and cell types including fibroblasts, endothelial cells, and immune cells.[1–3] These components of the tumor stroma contribute to the development of cancer by influencing the growth, progression, and metastasis of small tumors.[2,4,5]

Many studies of the cell biology of cancer rely on monolayer (2D) monocultures.[6–10] This type of in vitro culture does not recapitulate the complex environmental signals, interactions, and processes that characterize a tumor and its environment.[11] There is, thus, a need for physiologically relevant cancer models that include multiple cell types and that model the dynamic interactions between stromal and tumor cells. While there are many 3D systems available to study cocultures, these are limited to a small number of configurations, use complex instrumentation, or require multistep procedures.[3,12,13] In addition, most of these systems cannot enable generation of physiologically relevant structures that resemble tumors because they cannot mimic the limitations (e.g., mass transport) to the supply of O_2, nutrients, and signalling factors.[3,10,14]

The objective of our work is to develop experimentally and conceptually simple, flexible scaffolds and systems with which to study the behavior of tumor cells in 3D cocultures. We hope these systems will better recapitulate in vivo tumor biology than more familiar monolayer (2D) culture of tumor cells and transwell systems.[15–17]

We used a multilayered, paper-based platform (which we call "cells-in-gels-in-paper" or CiGiP) to coculture primary human lung tumor cell lines (VXN2s) and tumor fibroblasts (TFs) that were isolated from surgical tumor resections. We cocultured these human lung cancer cells and fibroblasts in Matrigel within the stacks of 100 μm thick-paper scaffolds (**Figure 1**). We varied the locations of the two cell types in order to study patterns of cell migration.

We found that the human lung cancer cells migrated toward an adjacent layer containing fibroblasts when it was included in the stacked layers of gel (and cells) supported in paper. The migration of the tumor cells increased upon coculturing them with fibroblasts, an observation consistent with examples from the literature.[18] We suggest that this behavior could, in part, be due to the activity of the transforming growth factor-beta (TGF-beta) pathway. Literature examples have previously demonstrated that the TGF-beta pathway influences the migration and metastatic behavior of cancer cells.[19,20] We therefore inhibited TGF-beta, and monitored the resulting effects on the migration of cells. Blocking TGF-beta decreased the number of tumor cells that were found in the fibroblast-containing layers.

Epithelial-to-mesenchymal transition (EMT) is a process by which the tumor epithelial cells lose their polarity and gain migratory properties.[21] The epithelial cells acquire mesenchymal features when the cells undergo EMT.[22] To determine the status of the lung tumor cells, we assessed their EMT behavior. We found that the inhibition of TGF-beta decreased EMT for the tumor cells.

The CiGiP approach provides a versatile tool for exploration of the areas of fundamental cell biology and development of new therapeutics. The multilayered paper constructs enable combining distinct types of cells in different patterns and configurations as well as high-throughput preparation and analysis of the samples in parallel. Stacking the layers of paper impregnated with cells in gel matrices easily assembles them into a 3D construct with control over the distribution of the cells. The modular paper constructs permits us to generate in vitro tissue-like structures through straightforward fabrication. This paper-based cell culture platform is simple, tunable, cost-efficient, requires only small amounts of samples, (i.e., cells, materials for gels, medium), and allows coculturing different types of cells. Using the CiGiP strategy allowed us to eliminate the cutting and sectioning steps in sample analysis and generate physiologically relevant constructs of tumors in 3D.

VXN2 cells were grown in both mono- and coculture configurations in the paper scaffolds within multilayered stacks. The cell-containing paper scaffolds were cultured for three days. We removed the samples from the holders, destacked the layers, and analyzed them to determine the percent (%) of VXN2 cells that are present in each layer of the scaffolds (**Figure 2**a).

We analyzed the experimental data using GraphPad Prism (Version 4.02, La Jolla, CA). The statistical differences between groups were determined by one-way ANOVA tests using Bonferroni comparisons. We considered the *p*-values that are smaller than 0.05 as statistically significant. The *p*-values were

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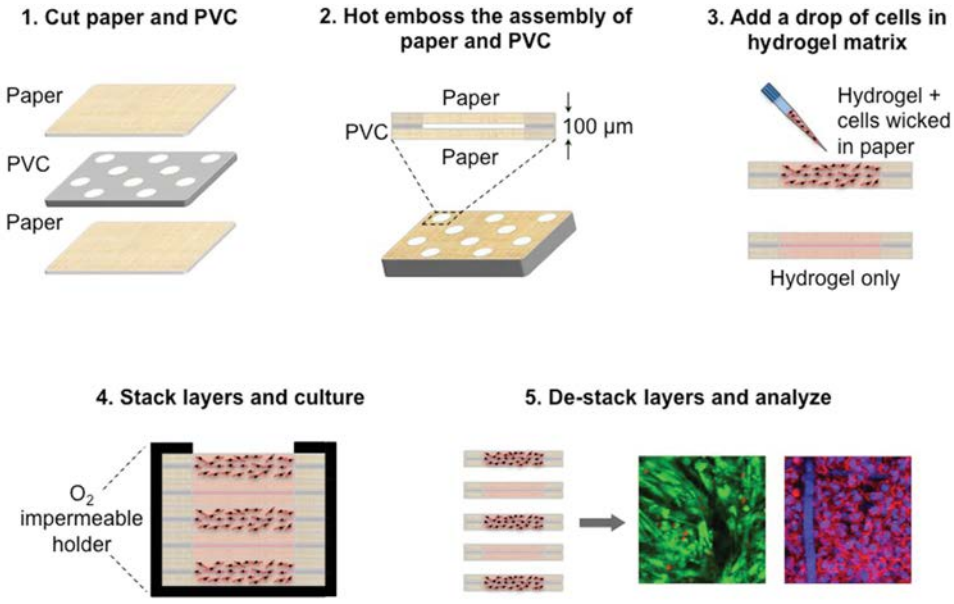

Figure 1. Schematic illustration for the cocultures of human lung tumor and fibroblast cells in the paper-based system. The cells were suspended in Matrigel solution and the resulting mixture was seeded on each culture zone of the double-lens paper. The cell-laden scaffolds were then stacked in a custom-made holder and cultured for three days to study migration of the lung tumor cells in the presence and absence of tumor fibroblasts in different configurations. Following the culture period, the paper scaffolds can be easily destacked and individually analyzed using a variety of analytical methods including toxicity, immunocytochemistry, spectroscopy, and microscopy.

defined by GraphPad Prism as follows: $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

Figure 2b–d demonstrates the results from the monocultures of human lung tumor cells in different layers of the paper stacks. In Figure 2b, VXN2 cells were cultured in Layer 1 (L1). In this configuration, the tumor cells did not migrate significantly beyond L4. Less than 2% of the cells were able to migrate to the bottom of the stack (L7). In Figure 2c, VXN2 cells were cultured in the middle layer (L4). The cells migrated to all of the layers within the stack. Migration of VXN2 cells to L3 was greater than to L5; we speculate that this difference reflects the higher availability of oxygen and nutrients in L3 than in L5. While we did not measure the amount of oxygen in different layers of the stack in this study, our group has previously demonstrated the presence of gradients of oxygen within stacks of paper scaffolds.[23] In the previous work, we have shown that cells exhibit different behaviors in migration when there is a gradient of oxygen. In Figure 2d, VXN2 cells were cultured in the bottom layer (L7). The tumor cells migrated all the way to L1, although from L5 to L1 migration was <2%.

VXN2-TF cocultures were designed to demonstrate the effect of fibroblasts on the migration of tumor cells within the stacked layers of paper. The results indicated that a subset of VXN2 cells migrated toward the layers that were occupied by the TF cells. In **Figure 3**a, VXN2 cells were cultured in L7 and TF cells, or no cells, were cultured in L1 and L2. There were higher numbers of VXN2 cells migrating to the layers containing TF cells compared to the same layers without TF cell culture.

VXN2 cells were cultured in the middle of the stack and TF cells were placed either in the top two or bottom two layers of the stack to study the migration behavior of VXN2s, in Figure 3b. We found that the VXN2 cells migrated toward the layers where the TFs were cultured, similar to the previous configuration in Figure 3a. We detected an increase in the number of migrating VXN2 cells as a result of seeding TFs in either the top or bottom layers. This observation suggests that the direction of migration has little bias resulting from position in the stack. Figure 3c summarizes the results from a coculture in which VXN2 cells were initially in L1, and the TFs were in L3, L4, and L5. In this experiment, the VXN2 cells migrated toward the layers that were occupied by the TF cells. Two possibilities might explain this observation: (i) VXN2 cells might be attracted to TF cells through their cross-talk, or (ii) VXN2 cells might proliferate more rapidly once they reach the layers that contain TFs.[24,25] In Figure 3d, VXN2 and TF cells were cultured in L1 and L7, respectively. The number of VXN2 cells migrating toward L7 containing the TF cells was greater than those without the TF cells. In behavior similar to those summarized in Figure 3c, the tumor cells might be attracted to fibroblasts as a result of paracrine signaling from the fibroblasts.[26] Proliferation of VXN2 cells, therefore, could also contribute to an increase in the number of cells in L7, over and above that reflecting the paracrine signals that are secreted by the cells when they come into contact.

Another reason for the increased migration of tumor cells in the presence of TFs might reflect the activity of the TGF-beta pathway. To test whether the VXN2 and TF cells secrete TGF-beta, we performed an ELISA assay (Figure S1, Supporting Information). The results indicated that TGF-beta was secreted by VXN2s, TFs, and cocultured VXN2-TF cells in the paper stacks.

After confirming that the cells secrete TGF-beta, we used 25 µg mL^{-1} of anti-TGF-beta antibody (R&D Systems,

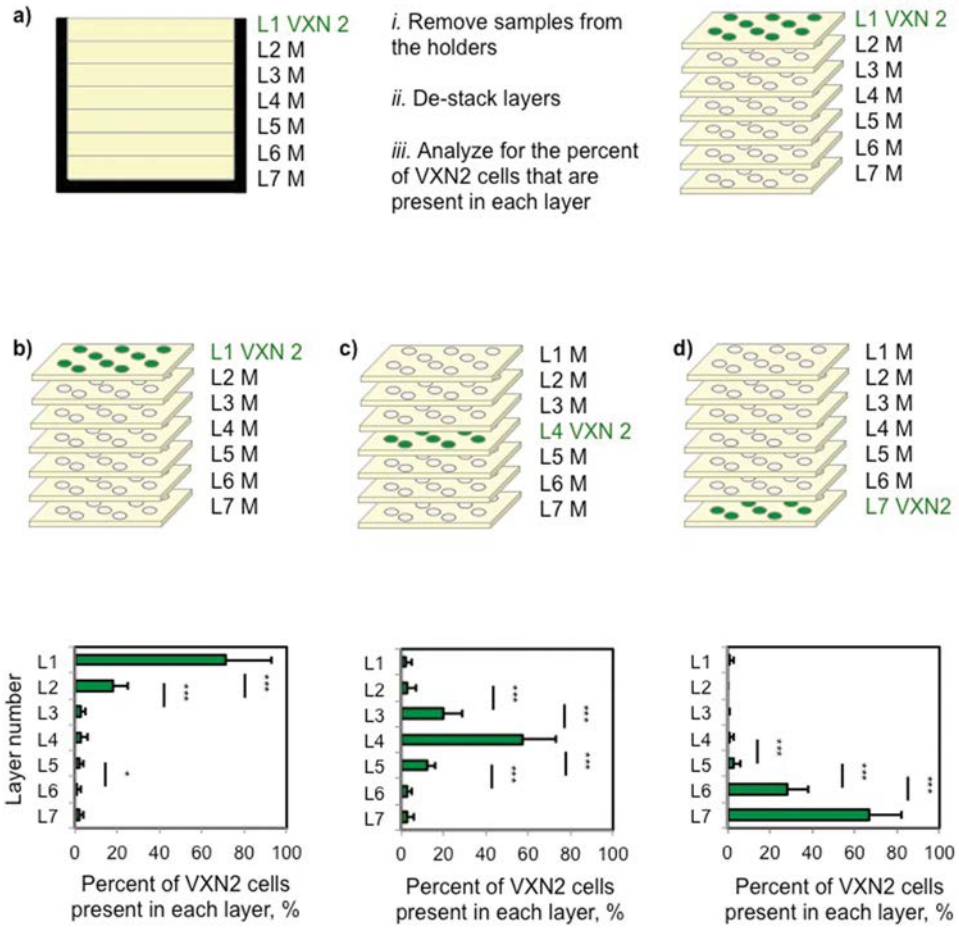

Figure 2. VXN2 lung tumor cells were grown in the multilayered paper scaffold system. VXN2 cells were monocultured in different layers of the paper stacks to determine their migration in the paper scaffolds. a) Following three days of culture, the samples were removed from the holders, destacked, and analyzed to determine the percent (%) of VXN2 cells that were present in each layer of the paper scaffolds. b) VXN2 cells were cultured in the top layer (L1). The results indicated that the tumor cells can migrate to the bottom of the stack, although migration was not seen at a significant extent beyond L4. c) VXN2 cells were cultured in the middle layer (L4). The cells migrated to the bottom of the stack. Migration of VXN2 cells was higher in L3 compared to L5 potentially due to the higher availability of oxygen and nutrients at the top of the stack. d) VXN2 cells were cultured in the bottom layer (L7), and migrated towards the top of the stack, including some cells that migrated up to L1. The statistical differences between groups were determined by one-way ANOVA tests using Bonferroni comparisons. We considered the *p*-values that are smaller than 0.05 as statistically significant. The *p*-values were defined by GraphPad Prism as follows: $*p < 0.05$ and $***p < 0.001$ ($n = 30$, error bars: ±SD).

AB-100-NA) to block the effects of TGF-beta in the coculture experiments. **Figure 4** displays the resulting plot. The VXN2 cells were cultured at the top layer (L1) and fibroblasts were placed at the bottom layer (L7). Blocking TGF-beta resulted in a significant decrease in the number of VXN2 cells that were found in L7 after 3 d. In order to test whether the activity of the TGF-beta antibody is specific to the antibody, we carried out a control experiment using antibodies against IgG. As expected, this control antibody did not influence the number of VXN2 cells resulting in the direction of the layers containing TFs. The migration pattern of the standard cocultures without the addition of an antibody was similar to that of the cultures with the control antibody.

To assess the metastatic potential of the VXN2 cells, we immunostained for the markers of EMT, E-Cadherin, and vimentin. **Figure 5** shows the confocal microscopy images. The mesenchymal status of the VXN2 cells is demonstrated through the expression of vimentin in the cocultures (Figure 5a). This observation is compatible with previous findings.[27] Tumor fibroblasts can stimulate the EMT and invasion of tumor cells through secretion of various cytokines. The cytokine TGF-beta, when secreted by tumor fibroblasts, can induce EMT of bladder tumor cells.[24] We used a TGF-beta neutralizing antibody and found that the inhibition of TGF-beta resulted in a decrease in the expression of vimentin in the VXN2 cells (Figure 5b).

Our results are consistent with literature examples. For instance, one study reported that lung colonizing potential of melanoma cells increased when they were cocultured with fibroblasts,[28] plausibly as a result of signalling factors that were released from the fibroblasts. Similarly, another study reported that the fibroblasts increased migration and invasion of colon cancer cells.[29] Another research group found that TGF-beta

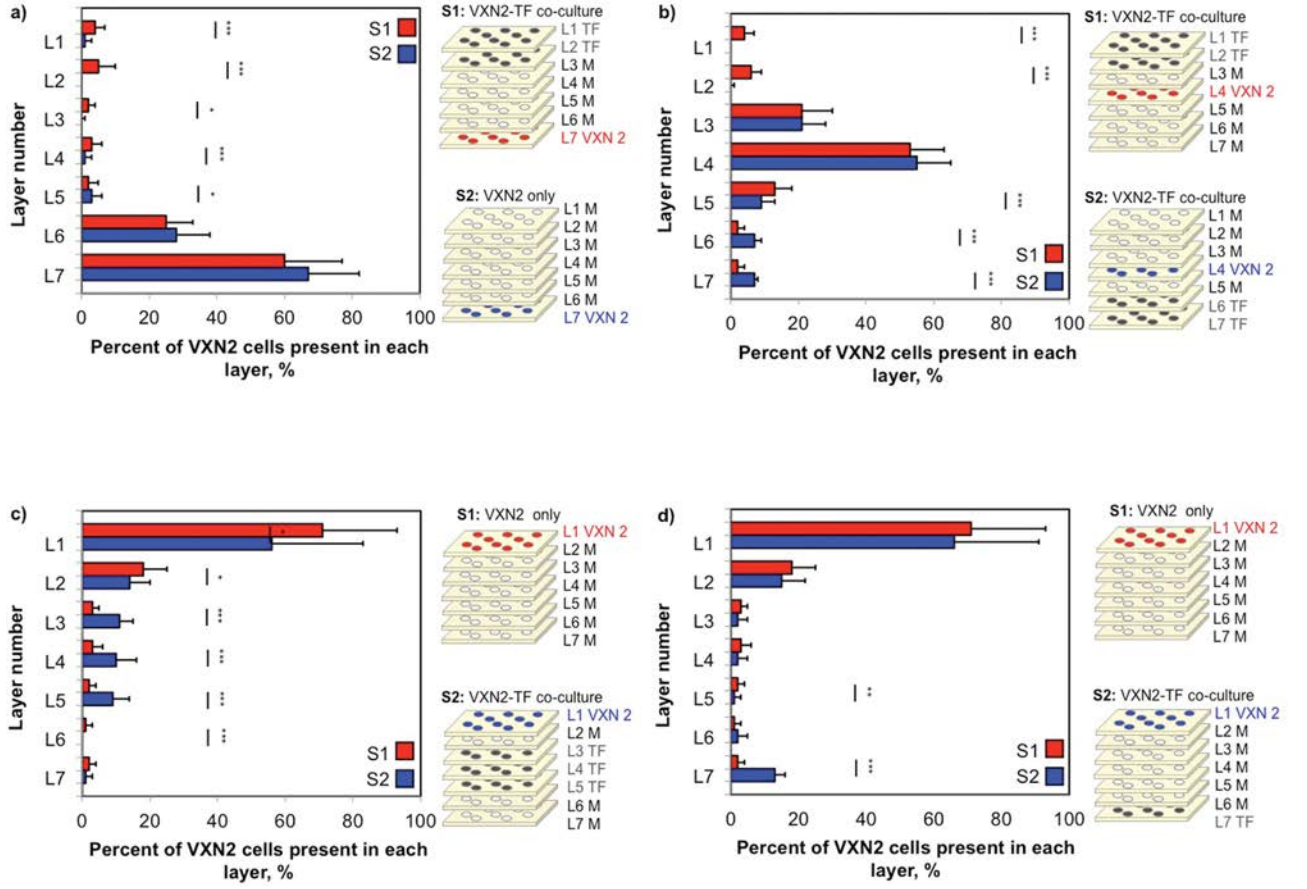

Figure 3. VXN2 and TF cells were cocultured in different layers of the paper stacks to study the migration behavior of tumor cells on day three. a) VXN2 cells were cultured in L7 in the first configuration. TF's, or no cells, were cultured in L1 and L2 and migration of VXN2s was compared. More VXN2 cells migrated to the top layers of the stack when there were TFs present. b) Cocultures of VXN2 and TF cells were compared in a different configuration. VXN2 cells were cultured in the middle of the stack and TF cells were placed either in the top or in the bottom of the stack. We found that the VXN2 cells migrated toward the layers where the TFs were maintained, regardless of TF position higher or lower in the stack. c) TF cells, or no cells, were seeded in L3-L5 while VXN2 cells were cultured in L1. Notably, VXN2 cells migrated to all the layers that contained the TF cells, despite less oxygen/nutrients toward the bottom of the stack. d) VXN2 and TF cells were cultured in L1 and L7, respectively. Similarly, more VXN2 cells migrated to L7 when TF cells were present than without TF cells. The statistical differences between groups were determined by one-way ANOVA tests using Bonferroni comparisons. We considered the *p*-values that are smaller 0.05 as statistically significant. The *p*-values were defined by GraphPad Prism as follows: $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ ($n = 30$, error bars: ±SD).

from fibroblasts increased the malignancy of metastatic breast cancer cells.[30]

The primary goal of this paper was to develop a simple paper-based system to study cocultures of tumor cells and fibroblasts. We used CiGiP for fabrication of multilayered stacked scaffolds to explore the migratory behavior of tumor cells in 3D. This approach may better recapitulate the dynamic interactions between tumor cells and fibroblasts compared to 2D cultures. We have established that tumor cells migrated more when they are cocultured with fibroblasts than when they existed as monocultures of tumor cells. We suggested that this finding could be partially relevant to the TGF-beta pathway, and found as a test of the hypothesis that when we inhibited TGF-beta, the number of tumor cells decreased. This reduction in the number of cells may be due to inhibition of migration, proliferation, or both.[31,32] TGF-beta, therefore, could be a factor participating in the migration process. In addition to migration, EMT is indicative of the metastatic potential of the tumor cells. We tested the VXN2 cells for EMT as a means to understand the metastatic potential of tumor cells in the presence of fibroblasts. We found that EMT decreased when TGF-beta was inhibited using an anti-TGF-beta antibody. This result is expected since TGF-beta inhibits migration as well as EMT and metastasis. We emphasize that we do not claim that the increase in the migration of tumor cells is exclusively or even primarily caused by TGF-beta. We suggest that our finding could, in part, be due to the TGF-beta pathway. The exact mechanisms of tumor cell migration in the presence of fibroblasts will require further study as will the possible contribution of TGF-beta to cancer cell proliferation in this system. The types of experiments that can be performed with the stacked multilayered paper include (i) investigation of the interplay between different types of stromal cells (e.g., immune, endothelial, connective tissue)

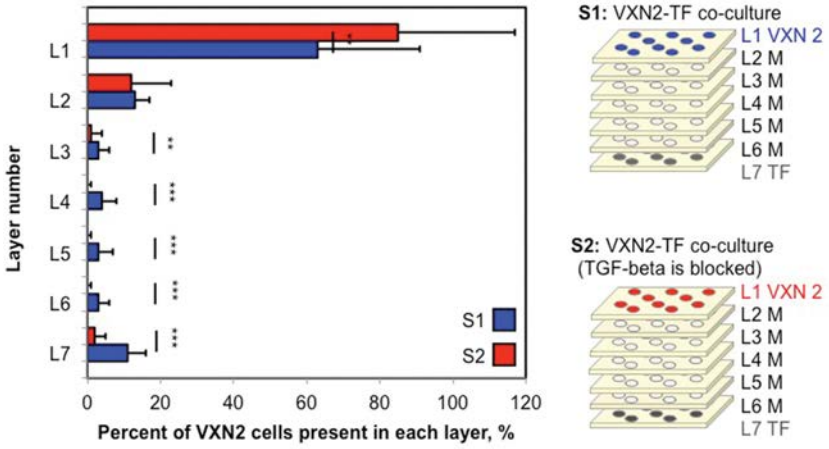

Figure 4. An antibody was used to block TGF-beta in the cocultures of VXN2 and TF cells in the paper-based platform. There was a significant decrease in the migration of VXN2s towards the bottom layers of the stack upon inhibition of TGF-beta. An IgG antibody was used as a control to demonstrate that addition of a non-specific antibody does not influence the migration of the cells ($n = 30$, error bars: ±SD, $^{**}p < 0.01$, and $^{***}p < 0.001$).

and tumor cells, (ii) decoupling the effects of oxygen from soluble factors of fibroblasts, and (iii) exploration of the influence of TGF-beta from different types of cells on the migratory behavior of tumors. The assessment of antimigration therapeutics could be another useful application of the stacked paper-based platform. The ability to prepare and analyze samples in a high-throughput fashion makes the stacked paper platform broadly useful for generation of physiologically relevant tumor models and development of new drugs. Paper can also be chemically modified to introduce bioactive functional groups that interact with cells in specific ways offering new opportunities for future studies.

The paper-based cell culture system is simple, flexible, inexpensive, and convenient to use for studying dynamic interactions between physiologically relevant types of

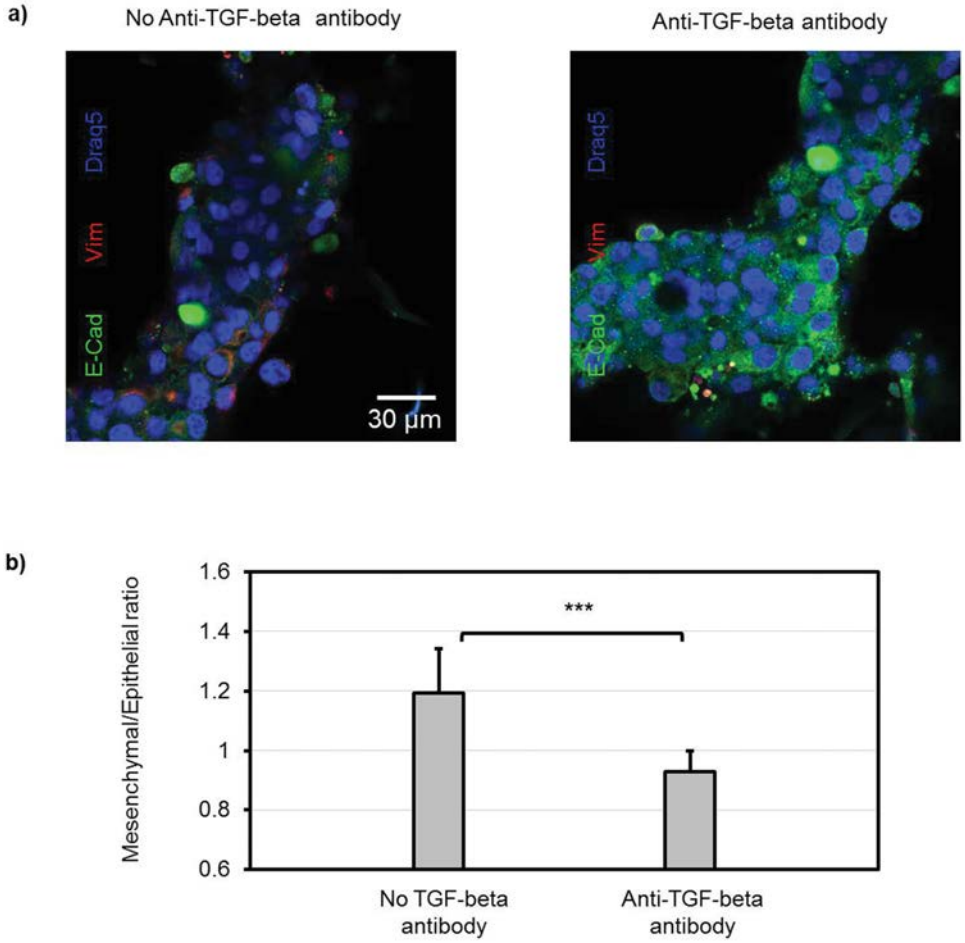

Figure 5. Immunocytochemistry experiments were performed to test for epithelial-to-mesenchymal transition (EMT). a) The transition was determined by staining for E-Cadherin (green) and vimentin (red). The nuclei were counterstained with Draq5 (blue). An antibody was used to block TGF-beta. The results demonstrated that blocking TGF-beta decreased the EMT for the VXN2 cells. b) The staining results were quantified to provide the differences between cases ($n = 10$, error bars: ±SD and $^{***}p < 0.001$).

cells. The paper-based scaffolds made it possible to generate physiologically relevant cancer models that include multiple cell types, and that model the dynamic interactions between tumor and stromal cells.

The paper-based tumor model also has several shortcomings. The lens paper is only 40 µm in the z-direction, therefore, may not be suitable for generation of constructs that would require significant mechanical strength. If an experiment utilizes the types of paper that are thicker than 200 µm in the z-direction, fluorescent imaging can be challenging due to scattering of light. In this work we cocultured tumor cells and fibroblasts; we can, however, include other cells such as immune and endothelial cells in order to better mimic the native microenvironment of tumors.

We believe that understanding 3D cocultures of cancer cells and fibroblasts will contribute to understanding the interactions of these cell types in tumors. The paper-based coculture platform may be useful for screening the inhibitors of TGF-beta pathway and perhaps investigating other stromal interactions that might suggest targets for therapy.

CiGiP also has the potential for use in personalized medicine. For example, patient biopsies might be harvested and grown to sizes relevant to vascularization and necrosis in this system. Different chemotherapeutic agents, or doses of radiation, could then be tested to determine effective treatment strategies specifically designed for the patient. The paper-based coculture approach simplifies the concept of patient-specific drug testing by miniaturizing the clinical trial in a dish, and by extending it in a new way into three dimensions.

Experimental Section

Preparation of Paper Scaffolds: We use paper as a scaffold to support thin layers of hydrogels containing cells; a number of prior applications of the method (CiGiP) are described elsewhere.[33–36] For applications in cell culture, the paper should have sufficient mechanical strength to hold the gel matrix containing the cells. In addition, it should have pore sizes that are large enough to support cell growth, spreading, elongation, and migration. For this work, we initially explored lens paper. Lens paper has large pore sizes (20–200 µm), but because it is only 40 µm thick in the z-direction, it is challenging to handle a single layer of lens paper in cell culture applications. We have, therefore, fabricated hybrid scaffolds that comprise two layers of lens paper sandwiching one layer of poly(vinyl chloride) (PVC). The resulting scaffolds are thick enough to easily handle the cell cultures and have pores sufficiently large to allow migration of cells.

Cocultures of Human Lung Cancer Cells and Tumor Fibroblasts in Multilayered Paper Stacks: VXN2 cells were initially monocultured at different layers in the paper stacks to explore their migratory behavior. As reported previously, these and other monocultures, do not adequately represent the environment that is important for growth of cells.[36–38] Monocultures, additionally, do not exhibit in vivo physiological behavior of cancer cells properly.[39] To provide an improved mimic of natural tumors, VXN2 and TF cells were grown as cocultures in multilayered paper stacks with different configurations.

Inhibition of TGF-Beta: To explore the influence of the activity of TGF-beta on migration of tumor cells, we used an antibody (R&D Systems, AB-100-NA) against it to block its effects. It is important to test a control antibody with the same isotype for validation of the results. We used an IgG antibody (R&D Systems, AB-105-C) and showed that the control antibody did not influence the migration of the tumor cells. We, therefore, concluded that the number of tumor cells was decreased due to the addition of TGF-beta blocking antibodies.

Epithelial-to-Mesenchymal Transition (EMT): EMT is a phenomenon by which the epithelial cells become migratory and invasive by transitioning into the mesenchymal phenotype. According to literature reports, EMT is involved in initiating the events for the metastasis of tumor cells into blood vessels, lymph nodes, or other organs.[40] The metastatic potential of a tumor is routinely analyzed by testing for the epithelial and mesenchymal phenotypes of the tumor cells.[41] The mesenchymal state is high in vimentin and low in E-cadherin, whereas the epithelial state is high in E-cadherin and low in vimentin.[42] We stained for E-cadherin and vimentin expression of VXN2 cells, and used the ratio of vimentin to E-Cadherin, which may be considered as one measure of the metastatic potential of the VXN2 cells.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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[1] J. Zhang, J. Liu, *Pharmacol. Ther.* **2012**, *137*, 200.
[2] R. M. Bremnes, T. Dønnem, S. Al-Saad, K. Al-Shibli, S. Andersen, R. Sirera, C. Camps, I. Marinez, L.-T. Busund, *J. Thorac. Oncol.* **2011**, *6*, 209.
[3] K. Chwalek, L. J. Bray, C. Werner, *Adv. Drug Delivery Rev.* **2014**, *79*, 30.
[4] K. Räsänen, A. Vaheri, *Exp. Cell Res.* **2010**, *316*, 2713.
[5] D. Hanahan, R. A. Weinberg, *Cell* **2000**, *100*, 57.
[6] S. S. A. Qadri, J. H. Wang, K. C. Redmond, A. F. O'Donnell, T. Aherne, H. P. Redmond, *Ann. Thorac. Surg.* **2002**, *74*, 1648.
[7] J. Yatsunami, N. Tsuruta, N. Hara, S.-I. Hayashi, *Cancer Lett.* **1998**, *131*, 137.
[8] C.-Y. Park, J.-Y. Son, C. H. Jin, J.-S. Nam, D.-K. Kim, Y. Y. Sheen, *Eur. J. Cancer* **2011**, *47*, 2642.
[9] J. Lin, K. Xu, J. Gitanjali, J. A. Roth, L. Ji, *Biochem. Biophys. Res. Commun.* **2011**, *410*, 235.
[10] D. T. Leong, K. W. Ng, *Adv. Drug Delivery Rev.* **2014**, *79*, 95.
[11] M. L. Teoh-Fitzgerald, M. P. Fitzgerald, W. Zhong, R. W. Askeland, F. E. Domann, *Oncogene* **2013**, *33*, 358.
[12] Y. Hao, L. Zhang, J. He, Z. Guo, L. Ying, Z. Xu, J. Zhang, J. Lu, Q. Wang, *PLoS One* **2013**, *8*, e61754.
[13] Z. Xu, Y. Gao, Y. Hao, E. Li, Y. Wang, J. Zhang, W. Wang, Z. Gao, Q. Wang, *Biomaterials* **2013**, *34*, 4109.
[14] K. M. Yamada, E. Cukierman, *Cell* **2007**, *130*, 601.
[15] K. I. Hulkower, R. L. Herber, *Pharmaceutics* **2011**, *3*, 107.
[16] C. Decaestecker, O. Debeir, P. Van Ham, R. Kiss, *Med. Res. Rev.* **2007**, *27*, 149.
[17] F. Entschladen, T. L. Drell, K. Lang, K. Masur, D. Palm, P. Bastian, B. Niggemann, K. S. Zaenker, *Exp. Cell Res.* **2005**, *307*, 418.

[18] O. Bermudez, E. Hennen, I. Koch, M. Lindner, O. Eickelberg, *PLoS One* **2013**, *8*, e63226.
[19] Q. Zheng, A. Safina, A. V. Bakin, *Int. J. Cancer* **2008**, *122*, 78.
[20] Y. Fang, Y. Chen, L. Yu, C. Zheng, Y. Qi, Z. Li, Z. Yang, Y. Zhang, T. Shi, J. Luo, M. Liu, *J. Natl. Cancer Inst.* **2013**, *105*, 47.
[21] R. Kalluri, R. A. Weinberg, *J. Clin. Invest.* **2010**, *120*, 1786.
[22] L. Lionel, B. Alfonso, *Oncogene* **2005**, *24*, 7443.
[23] B. Mosadegh, M. R. Lockett, K. T. Minn, K. A. Simon, K. Gilbert, S. Hillier, D. Newsome, H. Li, A. B. Hall, D. M. Boucher, B. K. Eustace, G. M. Whitesides, *Biomaterials* **2015**, *52*, 262.
[24] S.-H. Kim, C. Choe, Y.-S. Shin, M.-J. Jeon, S.-J. Choi, J. Lee, G.-Y. Bae, H.-J. Cha, J. Kim, *Anticancer Res.* **2013**, *33*, 2001.
[25] M. Horie, A. Saito, Y. Mikami, M. Ohshima, Y. Morishita, J. Nakajima, T. Kohyama, T. Nagase, *Biochem. Biophys. Res. Commun.* **2012**, *423*, 158.
[26] K. De Veirman, L. Rao, E. De Bruyne, E. Menu, E. Van Valckenborgh, I. Van Riet, M. A. Frassanito, L. Di Marzo, A. Vacca, K. Vanderkerken, *Cancers* **2014**, *6*, 1363.
[27] Z. Junlong, L. Qun, S. Bing, H. Xiaojing, S. Lan, Z. Xi, H. Ruimin, Y. Jun, G. Hongqian, *Sci. Rep.* **2015**, *5*, 11924.
[28] H. Tanaka, Y. Mori, H. Ishii, H. Akedo, *Cancer Res.* **1988**, *48*, 1456.
[29] M. L. Henriksson, S. Edin, A. M. Dahlin, P.-A. Oldenborg, Å. Öberg, B. Van Guelpen, J. Rutegård, R. Stenling, R. Palmqvist, *Am. J. Pathol.* **2011**, *178*, 1387.
[30] C. H. Stuelten, J. I. Busch, B. Tang, K. C. Flanders, A. Oshima, E. Sutton, T. S. Karpova, A. B. Roberts, L. M. Wakefield, J. E. Niederhuber, *PLoS One* **2010**, *5*, e9832.
[31] N. S. Nagaraj, P. K. Datta, *Expert Opin. Invest. Drugs* **2010**, *19*, 77.
[32] L. S. Hee, A. R. Paek, Y. K. Sil, K. S. Hyun, L. S. Young, Y. H. Jin, *BMB Rep.* **2015**, *48*, 115.
[33] R. Derda, A. Laromaine, A. Mammoto, S. K. Y. Tang, T. Mammoto, D. E. Ingber, G. M. Whitesides, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 18457.
[34] R. Derda, S. K. Y. Tang, A. Laromaine, B. Mosadegh, E. Hong, M. Mwangi, A. Mammoto, D. E. Ingber, G. M. Whitesides, *PLoS One* **2011**, *6*, e18940.
[35] F. Deiss, A. Mazzeo, E. Hong, D. E. Ingber, R. Derda, G. M. Whitesides, *Anal. Chem.* **2013**, *85*, 8085.
[36] H. Kaji, G. Camci-Unal, R. Langer, A. Khademhosseini, *Biochim. Biophys., Acta Gen. Subj.* **2011**, *1810*, 239.
[37] N. K. Paschos, W. E. Brown, R. Eswaramoorthy, J. C. Hu, K. A. Athanasiou, *J. Tissue Eng. Regen. Med.* **2015**, *9*, 488.
[38] L. Goers, P. Freemont, K. M. Polizzi, *Interface* **2014**, *11*, 20140065.
[39] S. Pizzimenti, *J. Pediatr. Oncol.* **2014**, *2*, 40.
[40] J. H. Tsai, J. Yang, *Genes Dev.* **2013**, *27*, 2192.
[41] C. Palena, R. I. Fernando, D. H. Hamilton, *Oncoimmunology* **2014**, *3*, e27220.
[42] X. Chen, R. Halberg, R. Burch, W. Dove, *J. Mol. Hist.* **2008**, *39*, 283.