SHORT-RANGE PARACRINE INTERACTIONS REVEALED IN A COMPARTMENTALIZED CO-CULTURE SCREENING PLATFORM

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ABSTRACT

We investigate tumor-stromal paracrine crosstalk by using microfabricated comb substrates [1], which maintain two cell populations in separate compartments but at very close proximity. We screen for cell-specific changes in gene expression during co-culture and our data suggest that tumor-stromal paracrine interactions regulate cancer stem cell and epithelial-mesenchymal-transition (EMT) markers in the tumor population. More importantly we find evidence for short-range paracrine interactions that cannot be detected using traditional approaches such as conditioned media transfer or Transwell culture inserts.

KEYWORDS: Tumor, Stroma, Paracrine, Co-culture, Microfabrication, Short-range, Gene expression

INTRODUCTION

Interactions between cancer cells and stromal cells in the local microenvironment play an important role in tumor growth, progression, and metastasis [2], however the mechanisms that govern this process have yet to be fully elucidated. A key difficulty in studying these interactions in co-culture is achieving cell-specific gene expression readouts from within a mixed population. For example, in a mixed culture it is difficult to assess whether changes in gene expression are occurring in tumor cells, stromal cells, or both without the use of harsh separation techniques, during which the gene expression of the cells could be altered. Similarly, paracrine communication cannot be studied independently from contact-dependent effects in mixed co-culture. Compartmentalized culture methods like conditioned media transfer and Transwell inserts confine cell populations in separate compartments to allow for easy separation, but these systems may not detect paracrine communication that is only effective at high concentrations or short distances. In conditioned media transfer, the concentration of soluble factors that are secreted into the media being conditioned is greatly diluted over time via diffusion and, furthermore, the two cell populations only participate in unidirectional communication rather than active feedback communication. Transwell inserts, on the other hand, do allow for bidirectional signaling, but separate the cell populations by 1mm, a distance too far for short-lived molecules such as reactive oxygen species [3].

THEORY

In order to study cell-specific tumor-stromal crosstalk, while allowing easy separation of the populations and not excluding short-range or concentration-dependent communication, we utilize microfabricated comb substrates [1].

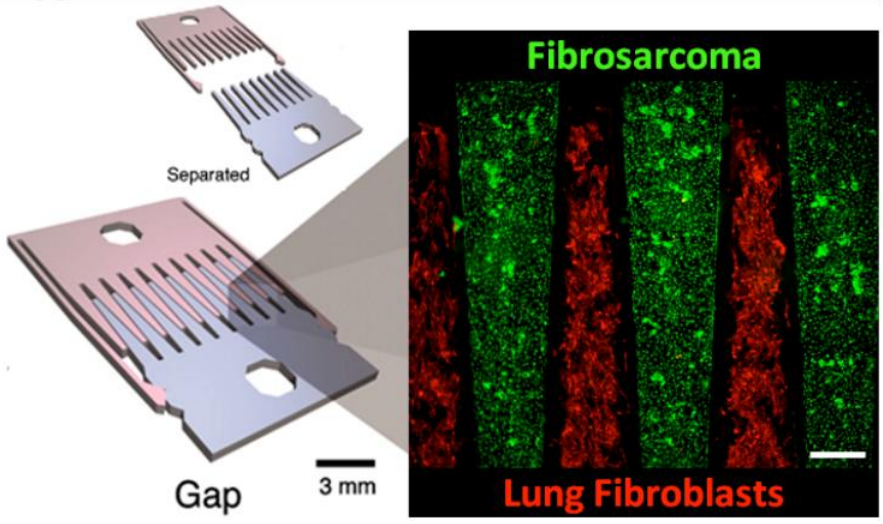

Figure 1: Microfabricated comb substrates. Devices are cut by silicon DRIE, coated with polystyrene, and plasma-treated to support cell adhesion. HT1080 fibrosarcoma cells (green) and stromal lung fibroblasts (red), shown here, are cultured in the gap mode with an 80 μm separation between the two cell populations. White scale bar is 250 μm.

These comb-shaped silicon plates are first coated with polystyrene and plasma treated to mimic cell culture plastic and then individually covered with opposing cell populations (e.g. tumor cells on one comb and stromal cells on another comb) and interlocked at a separation of 80 μm. This maintains the populations in separate compartments and thus allows for quick separation for population-specific assays, while still maintaining the cells in very close proximity so that short-range communication can occur. Some assays can be performed directly on the substrate (e.g. immunocytochemistry and other staining), while others can be performed on cell populations that were individually lifted off of the combs after co-culture (e.g. qPCR, Western blotting, flow cytometry).

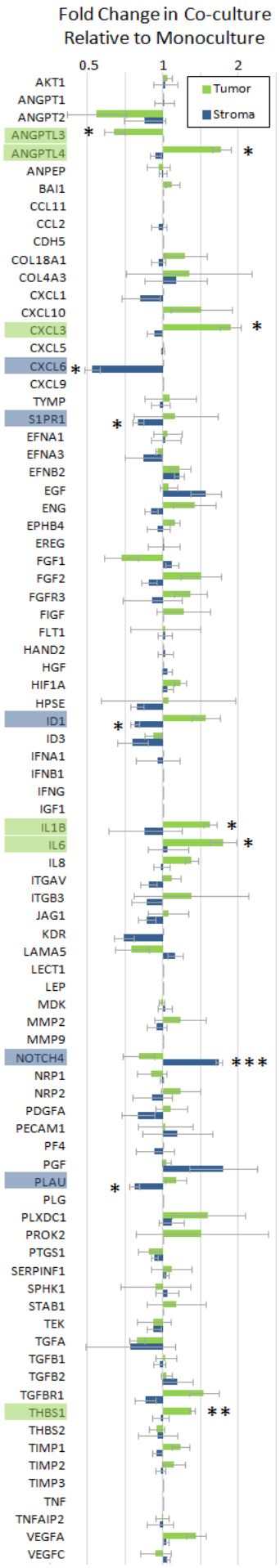

***Figure 2**: (Right) Gene expression changes detected by qPCR array. Following comb gap culture for 48 h, tumor and stromal cells were separated and individually subjected to qPCR array analysis. Fold-changes in gene expression relative to monoculture controls (n = 3) are plotted for each population individually, with tumor cell data in green and stromal cell data in blue. Error bars represent SEM. Significant changes in gene expression were determined using the Student's t-test. * = p<0.05, ** = p<0.01, *** = p<0.005. The name of genes showing significant changes in expression in a particular population are highlighted with the color representing that cell population.*

EXPERIMENTAL

Using the comb substrates, we co-cultured HT1080 fibrosarcoma cells and human lung fibroblasts (Fig. 1). This specific combination was used because the lung is a common site of fibrosarcoma metastasis [4] and we desired to study the tumor-stromal paracrine communication that occurs in a new metastasis. After 48 h in co-culture on combs, the cells were separated into pure populations and gene expression was measured in each using a qPCR array containing 84 genes associated with tumor progression. Significant changes in gene expression (n = 3) relative to monoculture controls were identified by the Student's t-test and those with low false discovery rates (FDR) and high fold changes were verified by conventional qPCR assay (n = 3). Similar experiments were also performed using conditioned media transfer and Transwell culture inserts, which are the two standard methods for studying cell-cell signaling between two separated cell populations. These were carried out with cells adhered to tissue culture plates, the traditional way, and with cells adhered to comb substrates, to control for substrate effects. Genes that exhibited significant expression changes by conventional qPCR assay in comb co-culture were also analyzed in samples from the standard co-cultures by conventional qPCR assay. Additionally, conventional qPCR assays were performed to measure gene expression changes in the cancer stem cell and EMT markers, Oct4 and αSMA.

RESULTS AND DISCUSSION

In the qPCR array assay, a number of genes from both populations exhibited significant changes in expression in comb co-culture compared to comb monoculture (Fig. 2). Many of these significant gene expression changes were validated by conventional qPCR (data not shown). Importantly, certain genes that were found to be significantly upregulated in comb co-culture showed no significant changes in standard co-culture formats.

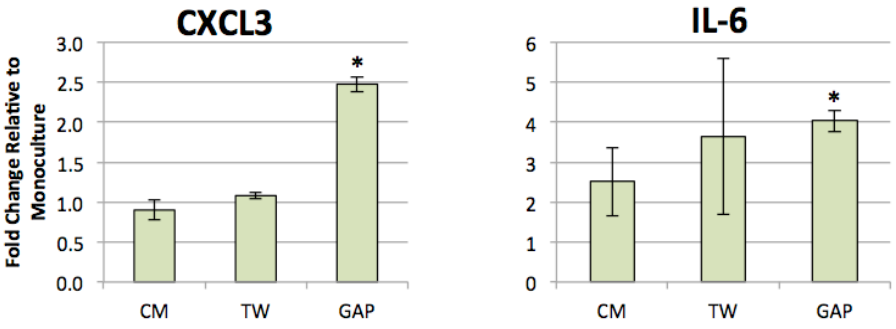

***Figure 3**: (Above) Short-range paracrine signaling. Induction of CXCL3 and IL-6 in tumor cells, presented as fold change induction in co-culture relative to monoculture controls. Data is shown for three co-culture configurations: conditioned media (CM), Transwell inserts (TW), and comb gap co-culture (GAP). CXCL3 induction is undetectable for conditioned media and Transwell cultures, but exhibits a 2.5-fold upregulation on the comb platform, suggesting evidence of short-range paracrine effects. For IL-6 induction, the short-range effect is not nearly as pronounced. Monoculture controls were performed on matched substrates (combs for GAP, and standard culture wells for CM and TW). Additional experiments where all three culture conditions were performed on comb substrates yielded identical results (data not shown).*

For example, expression of an inflammatory cytokine involved in migration, CXCL3, was increased by 2.5 fold in tumor cells during co-culture on comb substrates, but remained unchanged in the conventional cultures, in which the two cell populations were extensively further separated (Fig. 3). This result suggests that CXCL3 expression in tumor cells is regulated by a short-range paracrine interaction. An inflammatory cytokine involved in tumor cell survival, IL-6, was upregulated by 4-fold in tumor cells subjected to comb co-culture, but not significantly upregulated in the standard co-culture. This short-range effect, however, was not as pronounced. Additionally, Oct4 and αSMA expression was significantly increased in the tumor population from comb gap co-culture compared to comb monoculture suggesting that tumor-stromal paracrine communication in gap may promote cancer stem cell and EMT phenotypes.

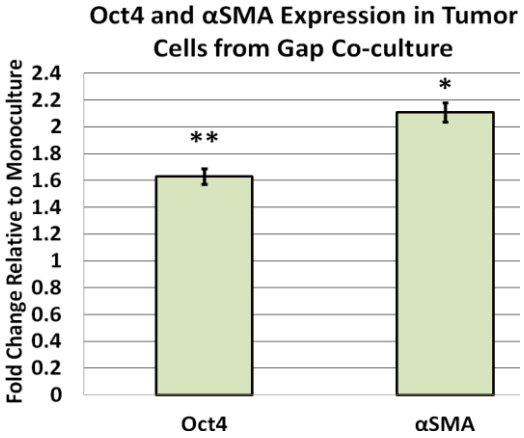

Figure 4: (Left) Upregulation of cancer stem cell and EMT markers. Significant fold changes in Oct 4 (1.62-fold) and αSMA (2.11-fold) expression was detected in tumor cells subjected to comb co-culture with stromal cells compared to tumor comb monoculture.

CONCLUSION

We demonstrate that comb culture substrates can be used in conjunction with high-throughput assays to study tumor-stromal interactions. Additionally, our data suggest that the comb platform may be sensitive to short-range paracrine effects that are lost when using conventional approaches. This finding bears important implications for the study of intercellular communication, as widely utilized co-culture techniques may be insensitive to an entire class of paracrine interactions, raising the possibility that important mechanisms of cell-cell signaling have been missed in previous studies. While our study was performed in the context of tumor-stromal interactions, the implications of this data apply broadly to cell-cell communication in general.

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