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RESEARCH LETTER

Epithelial-Stromal Interactions in Barrett's Esophagus Modeled in Human Organ Chips

Barrett's esophagus (BE) is an adaptive response of the lower esophagus to recurring exposure to gastroesophageal reflux that leads to intestinal metaplasia and/or gastric metaplasia depending on the specific criteria in each country.[1] The tissue microenvironment and local resident fibroblasts are critically involved in tissue homeostasis and repair processes[2]; however, the involvement of stromal-derived fibroblasts in BE and, in particular, their involvement in rare instances of metaplastic transformation and progression to esophageal adenocarcinoma (EAC) are poorly understood. To examine this, we have leveraged a human organ-on-a-chip (Organ Chip) microfluidic culture methodology[3] to construct tissue recombinant models containing esophageal epithelial cells isolated from organoids derived from multiple BE patients (Figure A1A and B and Table) interfaced with fibroblasts isolated from normal esophagus or from metaplastic, dysplastic, or cancerous regions of the same esophagus that was surgically resected from an EAC patient. Flow cytometric analysis confirmed that ∼80–95% of the stromal cells isolated from each of these regions stained positively for 2 different known fibroblast markers (CD90 and CD73), and that were regional differences as well as some interpatient variability in the expression of fibroblast surface markers, including CD36, podoplanin, platelet-derived growth factor-α, and platelet-derived growth factor-β (Figure A2A and B). Interestingly, this analysis also revealed that fibroblasts from a healthy, disease-free esophagus exhibited a distinct phenotype that those from adjacent normal-appearing regions from EAC patients. In contrast to a past in vitro BE modeling study,[4] all cell derivatives used in these chips were not genetically manipulated and their growth conditions were designed to retain the natural self-renewing property of the BE tissue which is believed to arise from esophageal or gastric glandular epithelium,[5–7] rather than using conditions optimized for growth of normal squamous epithelium. This is the first time, to our knowledge, that it has been possible to analyze in vitro the heterogeneous responses of BE epithelium to coculture with stromal cells from different regions of the same organ from the same patient that exhibit differences in disease phenotype in vivo.

The Organ Chips contain 2 parallel channels separated by a porous membrane and the top of the upper channel is open and can be closed with a replaceable cover.[8] The fibroblasts were cultured in a collagen gel in the open channel above the porous membrane and the epithelial cells were then overlayed on top of the cell-filled gel. Both channels were perfused with organoid growth medium for the first 4 days of culture before medium was removed from the top channel to create an air-liquid interface; organoid medium was then perfused only through the basal channel for the remaining 10 days of culture. Histological analysis revealed that while cocultures of healthy fibroblasts with esophageal epithelium isolated from cadavers without any known disease resulted in the formation of a normal stratified, squamous epithelium on-chip (Figure A1C), this was not observed in homotypic recombinants of BE epithelium and BE metaplastic fibroblasts. Instead of a squamous epithelium, the BE epithelium formed a continuous layer one to 3 cells thick that contained cells with diverse morphologies, including cuboidal cells and goblet-like cells, as well as cyst-like or closed glandular structures of varying size overlying the BE fibroblast-containing stroma (Figure 1A and B). Notably, when analyzed using a combined Alcian Blue and Periodic acid–Schiff stain, the cells within the epithelium exhibited various hues (Figure 1B), indicating a mix of intestinal (acidic) and gastric (neutral) mucins that recapitulates the variability observed in metaplastic

Table. Human Subject Information

Patient code	Patient diagnosis	Histopathology of tissue region(s) used	Sex	Age	Cell derivatives obtained
Patient 13	BE, non dysplastic	Metaplasia	M	N/A	Epithelial organoids
E02	EAC	Metaplasia	M	64	Epithelial organoids
E07	EAC	Dysplasia, cancer	M	58	Fibroblasts
E21	EAC	Metaplasia	F	68	None (only tissue block)
E24	EAC	Adjacent-normal esophagus, metaplasia, dysplasia+cancer	F	86	Fibroblasts
E26	EAC	Adjacent-normal esophagus, cancer	F	71	Fibroblasts
AGJS190	Died from head trauma, otherwise disease-free	Healthy esophagus	M	18	Epithelial cells (squamous) and fibroblasts

BE, Barrett's esophagus; EAC, esophageal adenocarcinoma; N/A, not available.

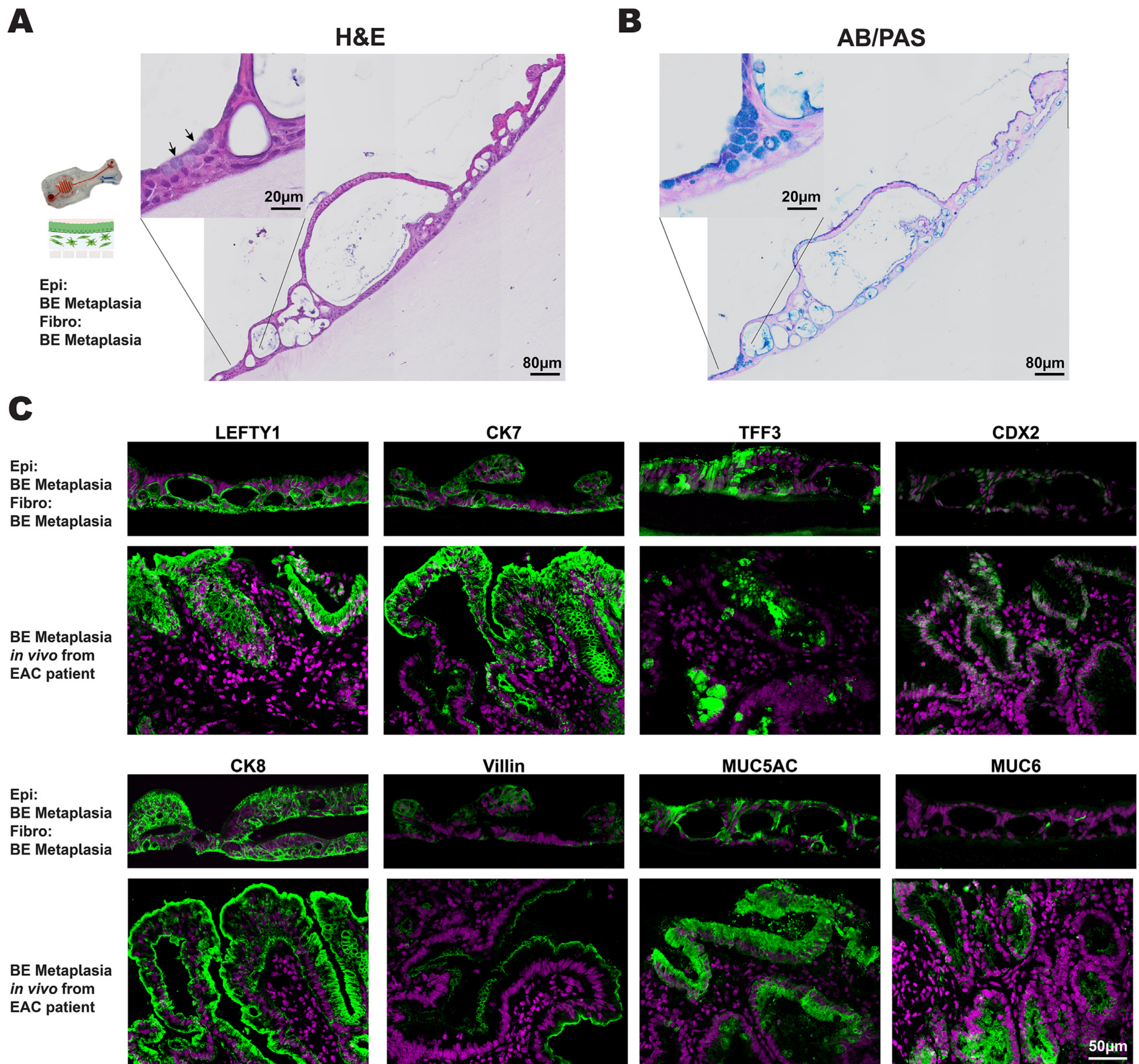

Figure 1. Establishment of homotypic tissue recombinants of human metaplastic epithelium and stromal fibroblasts isolated from patient-derived Barrett's esophagus (BE) resections and cultured in a two-channel, open-top, microfluidic Organ Chip. (A) Histological cross-section view of a paraffin section of a homotypic recombinant culture lined BE epithelium grown atop a type I collagen gel containing BE fibroblasts stained with hematoxylin and eosin (H&E) with an inset showing a smaller subregion at higher magnification. Arrow indicates epithelial cells with a goblet cell-like morphology. (B) A parallel section from A visualized using a combined Alcian Blue and Periodic acid–Schiff (AB/PAS) stain, with a similar inset. (C) Representative immunofluorescence microscopic views of cryosections of Organ Chips containing homotypic recombinants of BE epithelium and BE fibroblasts (top) vs views of sections of BE metaplastic tissues in vivo (bottom). The epithelial cells cultured within these BE Chips were stained for intestinal (CDX2, villin, cytokeratin 8, trefoil factor 3) gastric (MUC5AC, MUC6) differentiation, as well as proteins that have been previously shown to be specifically enriched in BE metaplastic regions (LEFTY1 and cytokeratin 7) in green with Hoechst stained nuclei in magenta. Note the similarity in staining patterns between the cultured BE Chip and the BE tissue in vivo. The sparsely distributed, unstained fibroblasts within the underlying ECM gel are not visible in these views.

lesions observed in BE patients.[5] Immunofluorescence microscopic analysis also revealed that these cells recapitulated the BE epithelial phenotype, as indicated by expression of cytokeratin (CK)-7 and LEFTY1, which have been previously shown to be characteristic of glandular epithelium in BE (Figure 1C).[6,9,10] They also displayed markers of intestinal differentiation, including CK8, villin, CDX2, and trefoil factor 3 (TFF3),[10] as well as the gastric differentiation marker Mucin 5AC, but not Mucin 6. Interestingly, the same epithelium did

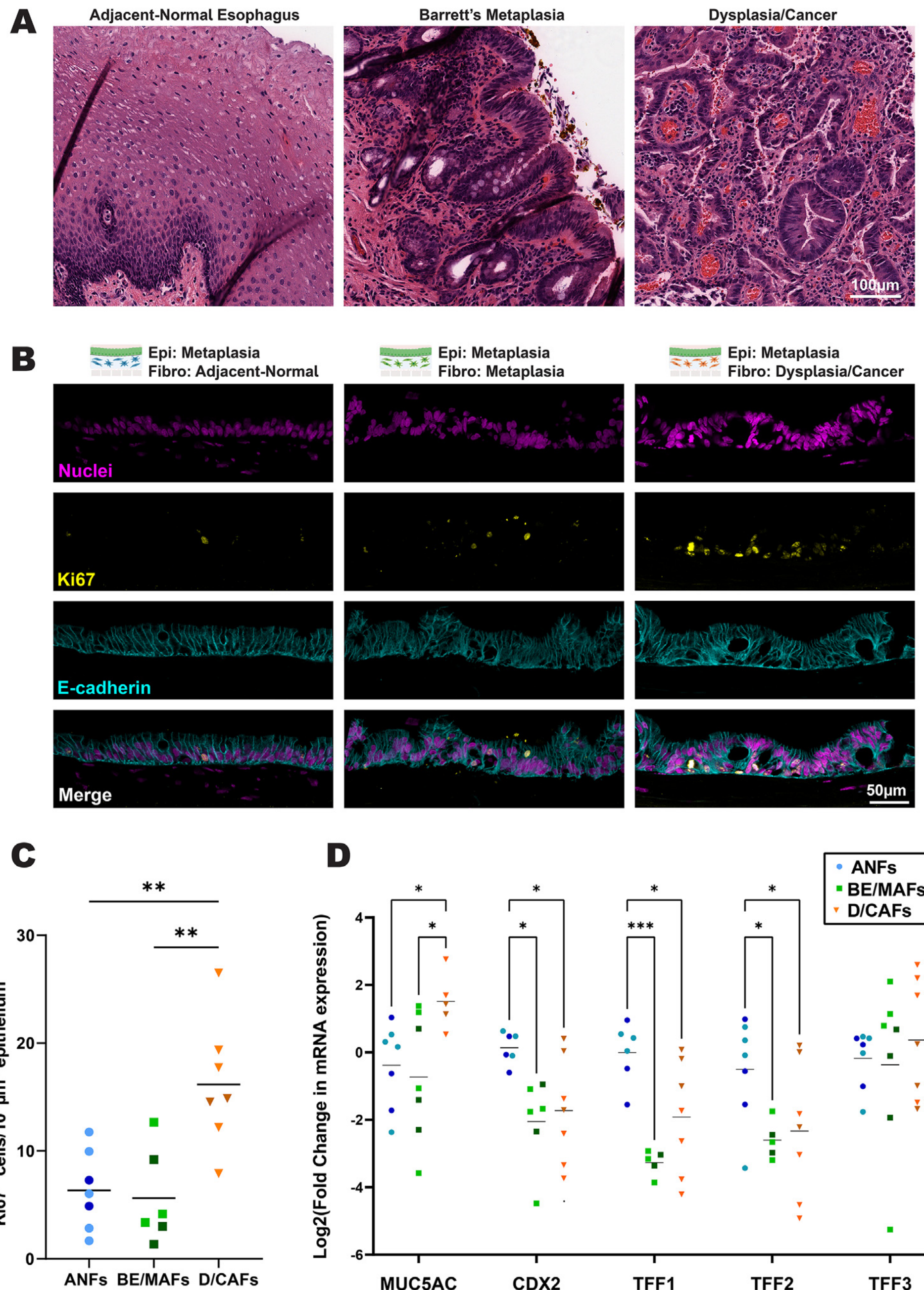

Figure 2. Organ Chips containing heterotypic tissue recombinants of human BE metaplastic epithelium with stromal fibroblasts isolated from adjacent normal, metaplastic, and dysplastic/cancerous regions from the same esophagus resected from an EAC patient. (A) Histological sections stained with hematoxylin and eosin (H&E) showing the morphology of the adjacent normal, metaplastic, and dysplastic/cancerous regions of the esophageal resection from EAC patient E24 that were used to isolate fibroblasts used to create the tissue recombinants on-chip. (B) Representative immunofluorescence microscopic views of cryosections of Organ Chips containing heterotypic recombinants of BE epithelium (from either patient E02 or patient 13) and adjacent normal fibroblasts (left), BE metaplastic fibroblasts (middle), and fibroblasts from the region that displayed both dysplastic and cancerous features (right), all isolated from the resected esophagus of EAC patient E24. Cell nuclei were stained using Hoechst, proliferating cells using anti-Ki67 antibody, and cell-cell junctions with E-cadherin antibody. The unstained fibroblasts are not visible in these views. (C) Graph showing the quantification of the number of Ki67-positive cells per project epithelial area in cross-sections of chips containing BE metaplastic epithelium combined with adjacent normal fibroblasts (ANFs), BE metaplasia-associated fibroblasts (BE/MAFs), or dysplastic/cancerous fibroblasts (D/CAFs) (**$P < .01$; each data point represents one BE Chip, different shades represent the 2 different epithelial donors, patient 13 (dark symbols) and E02 (light symbols). (D) Graph showing results of quantitative real-time PCR analysis of the effects of combining BE epithelium with ANFs, BE/MAFs, or D/CAFs on mRNA expression of selected genes related to gastric differentiation (MUC5AC, TFF1-2) and intestinal differentiation (TFF3, CDX2). Data were normalized relative to the mean value for the respective ANFs condition for each epithelial donor and are presented as log2 of the fold change (*$P < .05$, ***$P < .001$; different shades represent the 2 different epithelial donors, patient 13 (dark symbols) and E02 (light symbols).

not express TFF3, villin, or CDX2 when grown as organoids in static 3-dimensional gels (Figure A1B). Thus, this homotypic recombinant BE Chip that experiences dynamic fluid flow and an air-liquid interface as occurs in vivo replicates many of the histological features of metaplasia including the wide range of cell differentiation fates that is also observed in the esophageal epithelium of BE patients (Figure 1C).

Building on accumulating evidence that cancer-associated fibroblasts (CAFs) contribute to development of EAC,[11,12] we then engineered Organ Chips containing heterotypic tissue recombinants with BE epithelium overlying stroma containing fibroblasts from different regions of the diseased esophagus (Figure A3A) to compare how fibroblasts isolated from 3 different regions of a surgically resected esophagus from the same EAC patient might influence BE epithelial cell behavior. For these studies, we used fibroblasts isolated from regions that were determined by a pathologist to represent adjacent normal, BE metaplasia, or a mixture of dysplasia and cancer (Figure 2A). These studies revealed that heterotypic recombinants containing BE epithelium and dysplasia/CAFs (D/CAFs) from the more malignant region significantly increased epithelial cell proliferation on-chip compared to chips containing adjacent-normal fibroblasts (ANFs) or BE/metaplasia-associated fibroblasts (BE/MAFs), as indicated by Ki67 staining (Figure 2B and C). Real-time quantitative PCR analysis of these cultures did not reveal induction of any significant changes in expression of genes associated with intestinal differentiation (CDX2, TFF1, TFF2, TFF3) when D/CAFs were present, whereas MUC5AC that is expressed by differentiated gastric cells[13] was higher in these cultures (Figure 2D). In contrast, combination of ANFs with BE epithelium only resulted in higher levels of expression of intestinal differentiation markers (CDX2, TFF1, TFF2) compared to cultures with either BE/MAFs or D/CAFs (Figure 2D). These differences may indicate either a shift in the number of differentiated cells expressing these genes, or a reduction in the extent of differentiation and tissue-specific commitment or both. Finally, analysis of cytokines and chemokines present in the effluent of the lower channel of the chip showed that tissue recombinant cultures containing BE/MAFs secreted significantly higher levels of the collagenase matrix metalloproteinase-1 as well as the macrophage chemoattractant CCL2 (Figure A3B). This suggests that extracellular matrix remodeling and inflammatory signaling may be augmented in this homotypic BE model when comparing chips with either earlier stage ANFs or more malignant D/CAFs in the stroma. It is also consistent with the finding of a less dense collagenous stroma in chips containing BE/MAFs compared to healthy fibroblasts (Figure 1A and B vs Figure A1C). Interestingly, while the growth and cytodifferentiation features did not significantly differ between the tissue recombinants containing ANFs vs BE/MAFs with BE epithelium (Figure 2B and C), histological staining for nuclei and E-cadherin suggests that the presence of ANFs also induced a greater degree of epithelial polarization and somewhat normalized tissue architecture, as indicated by the consistent position of the nuclei at the base of epithelial cells that were primarily columnar in form as well as the relative absence of cyst-like structures (Figure 2B).

Taken together, these results show that Organ Chip models created with patient-derived stromal cells representing different stages in BE progression to EAC, including adjacent normal, metaplastic, and mixed dysplastic and cancerous regions of the same esophagus can be used to identify stromal influences on esophageal epithelial cell behavior in vitro in a patient-specific manner. Clearly, this is currently an oversimplistic model, however, additional levels of microenvironmental complexity, such as different immune cells, selected extracellular matrix molecules, and vascular endothelium may be incorporated in this system to study the full range of heterogeneous stromal contributions to BE and EAC pathobiology in the future. This Organ Chip model of BE offers a new platform for studying epithelial-stromal interactions and broader underlying mechanisms associated with esophageal cancer progression, and potentially could serve as a tool for personalized drug-response assessments between different patients or genetic subpopulations.

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Supplementary materials

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Abbreviations used in this paper: **ANF, adjacent-normal fibroblast; BE, Barrett's esophagus; BE/MAF, Barrett's esophagus metaplasia-associated fibroblast; D/CAF, dysplasia/cancer-associated fibroblast; EAC, esophageal adenocarcinoma**

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Conflicts of Interest:
This author discloses the following: Donald E. Ingber is a founder, board member, and chairs the SAB of Emulate Inc., in which he also holds equity. The remaining authors disclose no conflicts.

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The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Data Transparency Statement:
Data and analytic methods are presented in the article and in the Supplementary Information section. Additional data are available upon request from other researchers by contacting the corresponding author.

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