Esophageal 3D Culture Systems as Modeling Tools in Esophageal Epithelial Pathobiology and Personalized Medicine

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SUMMARY
Pathology of the esophageal epithelium leads to perturbations of the normal proliferation-differentiation gradient. Three-dimensional culture of esophageal epithelial tissue provides a unique platform to study diseases of the esophageal epithelium. Herein, we examine various types of three-dimensional esophageal culture and discuss the future of these applications for translational research and personalized medicine.

The stratified squamous epithelium of the esophagus shows a proliferative basal layer of keratinocytes that undergo terminal differentiation in overlying suprabasal layers. Esophageal pathologies, including eosinophilic esophagitis, gastroesophageal reflux disease, Barrett’s esophagus, squamous cell carcinoma, and adenocarcinoma, cause perturbations in the esophageal epithelial proliferation-differentiation gradient. Three-dimensional (3D) culture platforms mimicking in vivo esophageal epithelial tissue architecture ex vivo have emerged as powerful experimental tools for the investigation of esophageal biology in the context of homeostasis and pathology. Herein, we describe types of 3D culture that are used to model the esophagus, including organotypic, organoid, and spheroid culture systems. We discuss the development and optimization of various esophageal 3D culture models; highlight the applications, strengths, and limitations of each method; and summarize how these models have been used to evaluate the esophagus under homeostatic conditions as well as under the duress of inflammation and precancerous/cancerous conditions. Finally, we present future perspectives regarding the use of esophageal 3D models in basic science research as well as translational studies with the potential for personalized medicine. (Cell Mol Gastroenterol Hepatol 2018;5:461–478; https://doi.org/10.1016/j.jcmgh.2018.01.011)

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Esophageal Stratified Squamous Epithelium: Structure and Physiological Function
As a hollow muscular organ, the esophagus serves the passage of ingested food and liquid from the oral cavity to
the stomach. Its luminal surface is lined by the mucosa, comprising stratified squamous epithelium and the underlying lamina propria and muscularis mucosa. The esophageal epithelium consists of proliferative basal keratinocytes and suprabasal keratinocytes, the latter undergoing postmitotic terminal differentiation, passive migration toward the luminal surface, and, ultimately, desquamation into the lumen. Through this dynamic process, a proliferation-differentiation gradient is generated while epithelial renewal occurs over a period of 2 weeks. Molecular markers defining basal keratinocytes include cytokeratins K5 and K14, transcription factors p63, and SOX2, and cell surface molecules such as neurotrophin receptor p75NTR, integrin β1 (CD29), integrin α6 (CD49f), and transferrin receptor (CD71). Suprabasal keratinocytes are defined by differentiation markers such as cytokeratins K4 and K13, involucrin, and filaggrin, coupled with down-regulation of basal keratinocyte markers.

Doupe et al proposed that the esophageal epithelium is maintained by a single population of basal keratinocytes that give rise stochastically to proliferating and differentiating daughters with equal probability; however, functional cell heterogeneity has been postulated among basal esophageal keratinocytes. A minor subset of basal keratinocytes divide slowly or rarely and may have properties of quiescent stem cells. Such a cell population may provide an explanation as to how premalignant keratinocytes accumulate genetic alterations over years without being lost through epithelial renewal. Multiple cell surface and functional markers have been suggested to identify unique subsets of basal keratinocyte stem/progenitor cells, including neurotrophin receptor p75NTR, integrins (β4, α6), and ABCG2 gene product. Esophageal keratinocytes expressing these molecular markers have shown colony formation and self-renewal capabilities while also generating terminally differentiated progenitor cells.

Species differences exist between rodents and human beings with regard to anatomic esophageal structure. Foremost, the rodent esophagus lacks esophageal glands and papillae, both of which are present in the human esophagus. In addition, the rodent esophagus shows more explicit keratinization in the superficial cell layers, also known as stratum corneum of the squamous epithelium, as compared with its human counterpart. The rodent stomach consists of 2 compartments: the forestomach and distal stomach, featuring squamous epithelium and columnar epithelium, respectively, and some regard the forestomach as the counterpart of the human lower esophagus. This is important to note because Barrett’s esophagus (ie, intestinal metaplasia of the esophagus) is a human mucosal lesion involving the esophagogastric junction and has been modeled at the squamocolumnar junction within the murine stomach.

One essential physiological function of the esophageal mucosa is to serve a barrier against thermal, physical, or chemical agents, and factors related to luminal contents, including microorganisms, food antigens, gastroduodenal acids, and alcohol, all of which may contribute to the pathogenesis of esophageal diseases. Unlike the stomach, duodenum, and intestine, the luminal surface of the esophagus is not densely covered by mucus layers. Given the lack of the stratum corneum in the human esophagus and the lack of esophageal glands in rodents, the epithelial barrier function of the esophagus is attributed mainly to intercellular junctional complexes including tight junctions, adhesions, and desmosomes formed by cell–cell adhesion molecules such as E-cadherin, p120 catenin, and Claudins. The dysfunction of these adhesion molecules has been implicated in esophageal disease conditions.

### Organ Culture and Multiple 3D Culture Models: What Are the Differences?

Throughout a long history of cell culture, various forms of 3D culture methodologies have been developed along with unique scaffolds, matrices, and cell culture media. In the esophagus, 3D culture systems have provided unique platforms to study multiple biological processes, including epithelial cell proliferation, differentiation, motility, stress response, and both homotypic and heterotypic cell–cell communications. Cellular interactions involve a variety of cell types (eg, fibroblasts, endothelial cells, and inflammatory cells) in the esophageal tissue microenvironment under homeostatic and pathologic conditions (eg, inflammatory milieu), and are mediated via cell surface molecules (eg, integrins and receptors such as Notch) as well as extracellular matrix proteins (eg, matrix metalloproteinases), as discussed in this review. The ability to experimentally manipulate 3D cultures has greatly enhanced our understanding of the molecular mechanisms and signaling pathways underlying esophageal physiology and pathophysiology.

Organ (explant) culture was a major tool for in vitro live esophageal tissue analyses before primary esophageal epithelial cell culture and esophageal cancer cell lines became available in late 1970s and early 1980s, respectively. The foremost advantage of organ culture is the maintenance of natural tissue architecture in situ. Organ culture may be used to study cross-talk between epithelial cells and nonepithelial cells in a live tissue-like context. Given the potential importance of a variety of cell types (ie, epithelial cells, fibroblasts, nerve cells, immune cells, and endothelial cells) present in the tissue microenvironment, organ culture indeed may be more physiologically relevant than other 3D culture systems because co-culturing multiple cell types remains difficult.

Early organ culture studies have shown that esophageal explants from animals and human beings remained viable for 3–14 days ex vivo and provided substantial insight into esophageal physiological functions, including secretory and absorptive activities by basal and differentiating prickle cells, as well as endocytosis mediated by prickle cells and terminally differentiated superficial cells. In fetal human esophagi, organ culture detected not only epithelial cell proliferation but also replacement of columnar ciliated epithelium with stratified squamous epithelium, recapitulating the epithelial changes occurring during esophageal development.
Organ culture also has been used to study esophageal pathologies. Production of esophagitis-relevant cytokines was shown in patient-derived squamous epithelial explants. Retinoic acid induced BE-like glandular differentiation in explants derived from squamous esophageal epithelium. Excised patient-derived mucosal biopsy specimens of Barrett’s esophagus showed increased proliferation and cyclooxygenase (COX)-2 expression in response to bile salt exposure. Likewise, acid pulse induced a hyperproliferative response in Barrett’s esophagus explants via altered Na+/H+ exchange. Radiation sensitivity was tested in tumor explants from ESCC and EADC.

Although the use of chemically defined medium containing insulin and hydrocortisone enabled the maintenance of live human esophageal tissues in organ culture for up to 6 months, most experiments in organ culture are performed for short time periods (24–72 h) only. This is in part because the proliferation kinetics of esophageal keratinocytes in organ culture are not necessarily reflective of those occurring in vivo. In addition, monolayer outgrowth of keratinocytes has been observed in explant culture. Organ culture is inevitably more complicated than the 3D culture systems discussed later. Other limitations of this method include the difficulty of genetic manipulation of human tissues and the inability to passage organ cultures. Esophageal explants have yet to be derived from genetically engineered mouse models. To this end, Cre-mediated gene deletion can be performed ex vivo in organ culture with tissues isolated from transgenic mice carrying tamoxifen-inducible cell type–specific Cre and a floxed gene of interest.

Unlike organ culture, most 3D culture systems involve dissociation of originating tissue samples or preparation of cells grown in monolayer cultures before 3D reconstitution in vitro. Such 3D culture systems can be largely classified into 2 categories. One category is represented by OTC in which esophageal epithelial cells are grown over a collagen matrix containing fibroblasts that mimics the subepithelial lamina propria. The second category features spherical 3D structures of esophageal epithelial cells typically generated under submerged conditions. This includes multicellular spheroid culture, sphere formation assays, and 3D organoids.

OTC, also known as raft culture, is a form of tissue engineering with recapitulation of esophageal physiology and pathology (Figure 1). OTC was first established in the field of skin biology in the 1980s when the dermal equivalent comprising contracted type I collagen and fibroblasts was submerged in liquid medium and used as a raft-like platform to grow multilayered epidermal keratinocytes at the air–liquid interface, mimicking skin architecture as found in vivo. The air–liquid interface triggers epithelial stratification and cornification by inducing terminal differentiation in the superficial cell layer with induction of a variety of squamous cell differentiation markers including cytokeratins, transglutaminase, involucrin, and filaggrin. Although normal keratinocytes generate well-organized epidermal architecture, keratinocytes transformed by oncogenic viruses such as human papilloma virus form disorganized epithelia displaying aberrant proliferation, differentiation, and invasion into the dermal compartment.

We adopted this system for normal and genetically engineered esophageal keratinocytes, optimizing culture conditions and evaluating the influence of a variety of sources of fibroblasts including the skin and the esophagus of adults, children, and embryos as well. Fibroblasts evaluated in OTC include those isolated from the skin and the esophagus of adults, children, and embryos, as well as cancer-associated fibroblasts. Although multiple esophageal cell lines were used successfully in OTC (Table 1), telomerase-immortalized normal human esophageal keratinocytes (EPC2-hTERT) and primary human fetal esophageal fibroblasts (FF-E3) have served as standard and quality-control cells. EPC2-hTERT cells show complete stratified epithelia in OTC. Of note, commonly used HET1A, an oncogenic simian-virus-40 T-antigen-immortalized human esophageal epithelial cell line, did not recapitulate normal squamous esophageal epithelium in OTC.

With detailed protocols published by Kalabis et al., we have interrogated gene and molecular functions as well as cell–cell and cell–extracellular matrix interactions in broad contexts related to the esophageal physiological and pathologic microenvironment as detailed later in this article.

In vivo transplant culture has served as another platform to reconstitute esophageal epithelium in 3D. According to this method, which may not be categorized as cell culture in a strict sense, epithelial cells and fibroblasts are injected into the devitalized and denuded trachea tube of rats, which then is further transplanted into immunodeficient mice. Esophageal keratinocytes are allowed to grow for 4 weeks inside the xenografted trachea and the resulting epithelial structure may be analyzed morphologically. Wang et al. used this system successfully to characterize the contribution of Hedgehog signaling to esophageal development and metaplasia with esophageal keratinocytes isolated from genetically engineered mice.

Multicellular spheroid culture emerged in the early 1970s, stemming from dissociation-aggregation experiments in which dissociated tissue-derived cells undergo aggregation and self-organization under free-floating conditions with gentle stirring via spinner flasks or roller bottles. This technique was applied to a variety of cell types with primary culture and established cell lines recapitulating histologic tissue architecture of the originating organ. This platform has been used to study cross-talk between tumor cells and other cell types (eg, immune cells and endothelial cells) in co-culture experiments. Although multicellular spheroid culture was performed with nontransformed bovine esophageal epithelial cells as well as ESCC cell lines (Table 1), these studies were limited to morphologic comparison or analysis of cell viability in resulting spheroids.

In the history of 3D culture, the importance of extracellular matrix components for self-organization of functional epithelial cells has been recognized by studies using mammary epithelial cells grown under floating conditions in type I collagen or Matrigel (Corning, Tewksbury, MA) rather
than on a plastic surface. Under such conditions, mammary epithelial cells showed not only polarity but also luminal formation with milk protein secretion, which occurred more efficiently in the presence of Matrigel compared with type I collagen. Andl et al embedded esophageal epithelial cell aggregates into either type I collagen or Matrigel (Table 1) to observe that cell migration and invasion from the resulting spheroids were coupled with loss of E-cadherin-mediated cell adhesion. They further identified activin in the tumor microenvironment as a regulator of spheroid growth and invasion.

Sphere formation assays gained popularity in the 2000s as an excellent tool to characterize stem cells from multiple tissue types, including tumors (see Weiswald et al for an excellent review regarding tumor spheres and other spherical cancer models). Sphere formation assays were first used to show proliferation, self-renewal, and multipotent capabilities of neural stem cells in serum-free...
| 3D culture system | Esophageal epithelial tissue/cell | Cell designation | Research focus | Reference |
|------------------|----------------------------------|-----------------|---------------|-----------|
| OTC              | Tissue/normal: m                 | FACS-purified keratinocytes | Stem/progenitor cells | 110       |
|                  | Normal: h                        | Primary keratinocytes | Epithelial reconstitution | 53,54     |
|                  | Immortalized cell line: h        | HET-1A           | Epithelial reconstitution | 111       |
|                  | Immortalized cell line: h        | EoE2-T           | Epithelial reconstitution | 112       |
|                  | Nontransformed and transformed   | EPC1-hTERT EPC2-hTERT and derivatives | Notch in squamous-cell differentiation and Notch | 41,56     |
|                  |                                  |                 | AKT in squamous-cell differentiation | 54        |
|                  |                                  |                 | EGFR in basal cell hyperplasia | 55,61     |
|                  |                                  |                 | EGFR, cyclin D1, p53, and Notch in malignant transformation | 55,113    |
|                  |                                  |                 | EGFR and p53 in ESCC invasion | 114       |
|                  |                                  |                 | EGFR and p120 in ESCC invasion | 115       |
|                  |                                  |                 | PI3K and p120 in ESCC invasion | 58        |
|                  |                                  |                 | Periostin and ESCC invasion | 116       |
|                  |                                  |                 | STAT1 and ESCC invasion | 61,117    |
|                  |                                  |                 | Wnt in ESCC invasion | 60,61     |
|                  |                                  |                 | Notch in ESCC invasion | 59        |
|                  |                                  |                 | IGFBP3 in ESCC invasion | 118       |
|                  |                                  |                 | Activin A in ESCC invasion | 108       |
|                  |                                  |                 | CD44 and ESCC invasion | 119       |
|                  |                                  |                 | Autophagy and EMT in ESCC | 120       |
|                  |                                  |                 | c-Met and p53 in ESCC invasion | 121       |
|                  |                                  |                 | Wnt and COX2 in columnar-cell differentiation | 57,62     |
|                  |                                  |                 | Cdx1, c-Myc, and Notch in columnar-cell differentiation | 122       |
|                  |                                  |                 | Modeling esophagitis | 123,124   |
|                  |                                  |                 | EMT in EoE | 17,125,126 |
|                  |                                  |                 | Epithelial barrier functions in EoE | 52        |
|                  |                                  |                 | ESCC invasion | 111       |
|                  |                                  |                 | ESCC invasion | 112       |
|                  |                                  |                 | p38 MAPK in ESCC invasion | 127       |
|                  |                                  |                 | Fibroblast HGF in ESCC invasion | 49        |
|                  |                                  |                 | Modeling Barrett’s esophagus, all-trans retinoic acid in columnar differentiation | 128       |
|                  |                                  |                 | Modeling invasive EADC, cancer-associated fibroblasts and periostin in EADC cell invasion | 50,51,111 |
| ESCC cell line: h| TE9, TE10, KYSE70                |                 |                | 12,107    |
|                  | OE21                             |                 |                | 129       |
|                  | TE12                             |                 | ESCC invasion | 134       |
|                  | TE7, TE12                        |                 | ESCC invasion | 108       |
|                  | CP-A, CP-B, CP-C, CP-D          |                 | EMT and Notch in ESCC | 130       |
|                  |                                  |                 | Modeling Barrett’s esophagus, all-trans retinoic acid in columnar differentiation | 130       |
|                  |                                  |                 | Common culture conditions for murine and human organoids | 96        |
| Barrett’s esophagus cell line: h | MFD-1, OE19, OE33, FLO-1 |                 |                |            |
| EADC cell line: h |                                  |                 | Stem/progenitor cells | 12,107    |
|                  |                                  | Bulk primary keratinocytes | Basal cell hyperplasia and autophagy | 129       |
|                  |                                  | FACS-purified keratinocytes | Alcohol and autophagy | 134       |
|                  |                                  | Bulk keratinocytes | Common culture conditions for murine and human organoids | 108       |
|                  |                                  | EPC2-hTERT and derivatives | Basal cell hyperplasia and Notch in squamous-cell differentiation | 130       |
|                  |                                  | Barrett’s esophagus | Human Barrett’s esophagus 3D organoids | 96        |
| Organoid         | Normal, disease tissues: m       | FACS-purified keratinocytes | Stem/progenitor cells | 12,107    |
|                  | Bulk or primary keratinocytes    |                | Basal cell hyperplasia and autophagy | 129       |
|                  |                                  |                | Alcohol and autophagy | 134       |
|                  |                                  |                | EMT and Notch in ESCC | 108       |
|                  | Bulk keratinocytes               |                | Common culture conditions for murine and human organoids | 130       |
|                  | EPC2-hTERT and derivatives       |                | Basal cell hyperplasia and Notch in squamous-cell differentiation | 130       |
|                  | Barrett’s esophagus              |                | Human Barrett’s esophagus 3D organoids | 96        |
Table 1. Continued

| 3D culture system | Esophageal epithelial tissue/cell | Cell designation | Research focus | Reference |
|-------------------|----------------------------------|------------------|---------------|-----------|
| Sphere            | ESCC cell line: h                 | KYSE70, KYSE140, KYSE150, KYSE520, TE1 | ESCC CSC      | 82,83,87  |
|                   |                                  | CE81T, CE146T    | ICAM1 in ESCC CSC | 80        |
|                   |                                  | TE4, TE8         | JARID1B in ESCC CSC | 88        |
|                   |                                  | YES-2, Eca109    | ALDH and ESCC CSC | 84,85,91  |
|                   |                                  | KYSK270, T.Tn    | miR-377 in ESCC CSC | 93        |
|                   |                                  | Eca10R, Eca9706  | miR-181b and STAT3 in ESCC CSC | 92        |
|                   |                                  | KATO-TN          | YAP1 in ESCC | 86        |
|                   | EADC cell line: h                 | OE33, JH-EsoAd1  | EADC CSC | 81        |
|                   |                                  | JH-EsoAd1        | YAP1 in EADC CSC | 86        |
|                   |                                  | JH-EsoAd1        | ALDH in EADC CSC | 89        |
|                   |                                  | OE33, OE19, FLO1, JH-EsoAd1 | Notch in EADC CSC | 90        |
| Organotypic sphere culture | Tissue/normal: m, h | Primary keratinocytes | Stem/progenitor cells | 109       |
| Multicellular spheroid/Aggregate culture | Normal (bovine) | Primary keratinocytes | Morphologic comparison with spheroids formed with other cell types and detection of cell death in the inner cell mass within the resulting spheroid structures | 72        |
|                   | ESCC cell line (h)                | OSC-1, OSC-2     | Confirmation of spheroid formation | 73        |
|                   | Immortalized cell line (h)       | EPC2-hTERT and derivatives | E-cadherin and cell adhesion | 75        |

NOTE. In OTC, primary human FEF3 and other fibroblasts (eg, cancer-associated fibroblasts) were used to form subepithelial collagen matrix. Extensive lists of fibroblast cell lines and epithelial cell lines validated in 3D OTC are available in Kalabis et al.52

FACS, fluorescence-activated cell sorter; h, human; HGF, hepatocyte growth factor; ICAM1, intercellular adhesion molecule 1; m, murine; MAPK, mitogen-activated protein kinase.

*aAll of esophageal origin.
medium supplemented with epidermal growth factor (EGF) and differentiation-inducing other growth factors. To form 3D spherical structures, a single cell or a small number \((<10^1-10^2)\) of cells are inoculated per well and allowed to proliferate under free-floating conditions. This permits cell fate determination by tracking cell lineage within resulting spherical 3D structures. Unlike multicellular spheroid culture, cell aggregation is deliberately avoided at the onset of sphere formation assays to ensure a proliferative expansion of single-cell derivatives. In cancer research, sphere formation was used to explore cancer stem cells (CSCs) or tumor-initiating cells in conjunction with a variety of putative CSC markers and evaluation of therapeutic sensitivity for such cell populations.

As summarized in Table 1, sphere formation assays have been used to characterize EADC and ESCC CSCs expressing a variety of markers, including CD54 (intercellular adhesion molecule 1), CD49f, CD44, CD271, CD90, and aldehyde dehydrogenase, either alone or in combination. Increased sphere formation corroborated CSC attributes such as chemoresistance, invasiveness, and tumorigenicity, and expression of stemness markers (SOX2, ALDH1A1, and KLF4) induced by transforming growth factor-β1. Sphere formation assays were used to document the requirement of H3K4 demethylase Jumonji/Arid1b (Jarid1b) in the maintenance of ESCC CSCs and the role of Hippo coactivator yes-associated protein 1-mediated transcriptional regulation of SOX9 in EADC CSCs. Sphere formation also has been used to test pharmacologic therapeutic effects upon putative esophageal CSCs. Metformin appeared to inhibit sphere formation by Aldehyde dehydrogenase (ALDH1) EADC CSCs by targeting phosphatidylinositol 3-kinase/AKT and mammalian target of rapamycin. Pharmacologic inhibition of Notch signaling by γ-secretase inhibitors impaired tumor initiation as well as sphere formation by EADC CSCs, and Notch appeared to regulate genes such as SOX2, which is essential in stemness. The plant-derived agent curcumin also was found to decrease the sphere formation capability of ESCC cell lines.

In sphere formation assays, microRNA miR-181b was implicated in signal transducer and activator of transcription-3 (STAT3)-mediated transcriptional regulation of CSCs, whereas miR-377-mediated regulation of CD133+ ESCC CSCs has been shown. It must be noted that sphere formation assays in the studies described earlier were performed with cell lines, but not cancer cells isolated from primary tumors. In 1 study, sphere formation assays failed to detect ESCC CSCs expressing CD44 despite the high tumor-initiating capability of these cells being validated in mice. In addition, high densities (10^4-10^5 cells/well) of cells were seeded to form spheres in many of the described studies while several studies failed to provide information as to how spheres were generated, precluding determination of whether resulting spherical 3D structures represented cell aggregates or single-cell-derivative products. Because this represents a deviation from the original neurosphere assay principle, data obtained in these studies must be interpreted carefully.

The 3D organoid system has emerged in the past several years as a robust tool in basic research with the potential for personalized medicine. 3D organoids have been defined as a miniature organ-like 3D structure derived from single cells or a small number of cell clusters containing stem/progenitor cells grown in basement membrane (ie, Matrigel) under submerged conditions (Figure 1). Overcoming the difficulty to culture intestinal cell types, Sato et al were successfully able to grow stem and progenitor cells from either isolated intestinal crypts or single-cell suspensions prepared from the small or large intestine into lobulated 3D structures containing crypt and villus compartments, the latter containing terminally differentiated secretory and absorptive cell lineages. By passaging dissociated primary structures to generate secondary 3D organoids, this system has been used to validate the self-renewal activities of putative stem cells. Because this can be performed using live tissue pieces from biopsy specimens or even frozen tissues, this novel cell culture method has been transformative with great potential to advance personalized medicine, for example, by testing the chemotherapeutic sensitivity of colorectal cancer–derived 3D organoids from individual patients. Intestinal organoids have been coupled successfully with genetically engineered mouse models of colorectal cancer, facilitating functional studies into the biology of this organ in the context of health and disease. Application of the 3D organoid system has been extended to a variety of cell types from digestive (intestines, stomach, liver, pancreas) and nonintestinal organs (eg, brain, lung, breast, kidney, prostate, and ovary) from both human and murine tissue sources as well as embryonic or induced pluripotent stem cells.

3D organoids have been cultured in Dulbecco’s modified Eagle medium:nutrient mixture F12-based serum-free medium supplemented with transferrin, selenium, ethanolamine, insulin, antioxidants, and vitamins. This medium is supplemented with pharmacologic agents, such as transforming growth factor-β kinase/activin receptor-like kinase inhibitor A83-01, the p38 mitogen-activated protein kinase inhibitor SB202190 and the rho-associated kinase inhibitor Y-27632 to facilitate the establishment of organoids. Besides Matrigel, addition of growth factors and hormones, including noggin, EGF, Wnt3A, R-spondin, and gastrin, to 3D organoid media provides essential niche factors present in the tissue microenvironment in situ. Unique niche factors may influence organoid formation differentially from different cell types as pioneered by Sato et al in intestinal cell types. For example, Wnt3A is essential for stem cell maintenance in colonic, but not small intestinal, 3D organoids, in mice in which withdrawal of Wnt3A facilitates differentiation in murine colonic organoids. Optimization of 3D organoid culture medium has broadened the cell types of human and rodent origin that can be grown successfully and passaged as 3D organoids.
3D organoids featuring the stratified squamous epithelium of the esophagus were first established from murine esophageal mucosa by DeWard et al.\cite{12} to investigate esophageal stem and progenitor cells. This group used medium similar to that used for human intestinal 3D organoids.\cite{34} We have used simplified medium components to generate single-cell–derived murine esophageal 3D organoids for multiple passages,\cite{12,105} indicating that certain agents and factors are dispensable for murine esophageal 3D organoid culture. The conditions that we have optimized were permissive for generation of murine 3D organoids from normal esophageal epithelium as well as chemical carcinogen-induced precancerous and ESCC lesions.\cite{106}

Jeong et al.\cite{107} showed successful generation of clonally formed spherical 3D structures from both murine and human esophageal mucosa in a method referred to as “3D organotypic sphere culture.” Although esophageal keratinocytes were suspended in Matrigel, this method used 2 different types of keratinocyte serum-free media to maintain undifferentiated progenitor cells and induce terminal differentiation. In addition, the esophageal keratinocyte-containing Matrigel compartment was placed atop a cell culture insert and exposed to the air–liquid interface, although the contribution of this exposure to sphere formation and/or differentiation was not discussed.\cite{107}

Among the earlier-described esophageal 3D cell culture systems, OTC and 3D esophageal organoids have been used most extensively to model epithelial physiological as well as pathologic conditions (Table 1). As a form of tissue engineering, OTC has been most broadly used in studies focusing on epithelial cell behaviors, including epithelial-stromal interaction after pharmacologic and genetic manipulations of either epithelial orstromal cells\cite{17,41,47–50,60,73,106,108–125} (see Kalabis et al.\cite{52} for detailed protocols and resources available). OTC has been better characterized using human esophageal cells as compared with cells of rodent origin, although this method is possible with the latter.\cite{108} Although OTC requires preparation of monolayer cell culture before epithelial and subepithelial tissue reconstitution in vitro, the 3D organoids system is initiated directly after dissociation of live tissues and has been applied to both murine and human esophageal cells (Figure 1). In the following section, we review several normal and disease conditions in which OTC or 3D organoids have served as modeling tools.

**Esophageal Normal Stem/Progenitor Cell Proliferation and Differentiation in 3D**

Because the esophageal 3D organoid system features single-cell–derived clonal expansion, establishment of a squamous cell differentiation gradient, and the maintenance of 3D structure upon serial passaging, this experimental platform has been used to test the self-renewal capability of putative esophageal stem cells. DeWard et al.\cite{12} have shown that esophageal basal keratinocytes defined by Sox2 expression comprise stem cells and transit-amplifying cells defined by distinct levels of cell surface CD73, α6 integrin (CD49f), and β3 integrin expression with differential 3D organoid formation capabilities. Jeong et al.\cite{105} showed that undifferentiated esophageal keratinocytes defined by positive/high CD49f and low CD24 expression show high 3D sphere formation capability where the transcription factor p63 appeared to regulate self-renewal and gene expression of basal cell markers. Giroux et al.\cite{104} identified a long-lived stem/progenitor cell population characterized by expression of keratin 15 in murine esophageal epithelium, which showed self-renewal, proliferation, and differentiation capabilities in 3D organoid assays. As has been postulated as a weakness in conventional neurosphere assays,\cite{75} organoid formation involves cell proliferation and, thus, may not necessarily determine quiescent stem cells. In addition, the frequency of stem cells detected by these assays may not necessarily be representative of that found in originating tissues, potentially owing to loss of subsets of cells during tissue dissociation and cell isolation. The proliferation-differentiation gradient has been analyzed in esophageal stem cell–derived 3D organoids using molecular markers of basal keratinocytes and differentiated suprabasal keratinocytes in the earlier-described studies.\cite{12,105,107,126} Because the most proliferative and undifferentiated basal-like (or basaloid) keratinocytes are present in the outmost cell layer of 3D organoid structures and less proliferative differentiated cells represent the inner cell mass, one caveat of this model system is that the former does not migrate toward the center of the 3D structure to become the latter, unlike generation of the differentiation gradient in vivo or in OTC. Namely, the proliferative stem/progenitor cells form first the inner cell mass, which undergoes terminal differentiation because organoids grow in an outward fashion, and, thus, the outmost cell layers represent the cells that were generated last.

OTC also was used to show that putative esophageal stem cells are capable of reconstituting stratified squamous epithelia in vitro\cite{107}; however, a limitation of the use of OTC for the assessment of esophageal stem cells is that this technique fails to generate single-cell–derived 3D structures. Esophageal epithelial cell proliferation and differentiation have been characterized extensively along with their regulatory signaling pathways in OTC using either primary human esophageal keratinocytes or telomerase-immortalized cell lines.\cite{41,47,48} Esophageal keratinocytes engineered to overexpress epidermal growth factor receptor (EGFR) showed hyperproliferation in an EGFR tyrosine kinase activity–dependent manner in OTC.\cite{47} This study showed that EGFR overexpression induces translocation of p120 catenin to the cell membrane, which mediates epithelial cell–cell adhesion.\cite{47} Among downstream effectors of EGFR is the phosphatidylinositol 3-kinase/AKT signaling pathway.\cite{127} In OTC, EGFR overexpression led to AKT activation via phosphatidylinositol 3-kinase. Although inducible activation of AKT increased cell size concurrent with a decreased level of keratohyalin granules, a marker of terminal differentiation, AKT activation did not increase esophageal cell proliferation in OTC.\cite{54} EGFR signaling regulates the cell cycle by inducing cyclin D1, a key G1 cyclin.\cite{128} Cyclin D1 was found to be responsible for basal keratinocyte proliferation because tetracycline-inducible cyclin D1 overexpression resulted in basal cell hyperplasia.
In OTC. The transcription factor KLF4 facilitates esophageal differentiation via activation of noncanonical Wnt5A signaling.

Notch signaling plays a critical role in esophageal epithelial cell fate decisions. In studies using OTC coupled with RNA interference, genetic or pharmacologic pan-Notch inhibition showed that activated Notch signaling drives terminal differentiation by transcriptionally activating early differentiation markers such as involucrin and cytokeratin K13 in a Notch3-dependent manner. These observations in OTC were recapitulated in genetically engineered mice with esophageal epithelium-targeted pan-Notch inhibition showing Notch3 down-regulation and impaired terminal differentiation. In our recent attempt to dissect Notch signaling in normal human esophageal 3D organoids, a similar approach confirmed the role of Notch signaling in basal keratinocyte exit toward terminal differentiation. Thus, the OTC and 3D organoid models may complement studies in genetically engineered mouse models.

**Impaired Epithelial Homeostasis and Barrier Defect Modeled in 3D**

A variety of stressors in the tissue microenvironment influence esophageal homeostasis. Little has been explored as to how esophageal epithelial cells respond to these stressors in 3D culture. In esophageal 3D organoids, we recently investigated epithelial response to oxidative stress induced by alcohol and its toxic metabolite acetaldehyde or inflammatory cytokines. These stressors were found to induce autophagy, a homeostatic cytoprotective mechanism to decrease oxidative stress, recapitulating epithelial changes observed in mice subjected to excessive alcohol drinking in the presence of dysfunctional Aldh2, a mitochondrial acetaldehyde metabolizing enzyme or EoE-like esophageal inflammation.

An epithelial barrier defect has been implicated in the pathogenesis of esophageal diseases including GERD and EoE. Squamous epithelial stratification is less mature and suboptimal in OTC lacking the subepithelial matrix compartment containing fibroblasts. Underscoring the cross-talk between stromal fibroblasts and epithelial cells in epithelial homeostasis, OTC-like air–liquid interface was used in the absence of fibroblasts to study the role of desmoglein-1, calpain 14, and LLRC31 in epithelial barrier functions. In these studies, genetic modulations of these molecules resulted in impaired epithelial stratification as corroborated by altered trans-epithelial resistance or dextran flux; however, the absence of fibroblasts in the OTC-like system may have potentially exaggerated the observed barrier defects because the reconstituted epithelia contained much fewer regular basal cells than the typical OTC with fibroblasts.

**Modeling Inflammatory Disease Conditions in 3D**

Inflammation is pertinent to multiple esophageal pathologies including GERD, EoE, radiation-induced esophagitis, as well as preneoplastic and neoplastic conditions; however, modeling inflammation in 3D culture systems remains largely unexplored. Laczko et al created an OTC esophagitis model by incorporating human peripheral blood mononuclear cells and exogenous cytokines into OTC, recapitulating the T-helper cell type 1 acute inflammatory response found in human GERD. Indeed, esophageal epithelial cells showed aberrant proliferation, differentiation, oxidative stress, DNA damage, and apoptosis in response to peripheral blood mononuclear cells and stimulation by cytokines in OTC.

EoE is characterized by long-term inflammation mediated by eosinophils and other immune cell types that lead to substantial tissue remodeling affecting both epithelial and subepithelial stromal compartments. In EoE, esophageal epithelial cells show basal cell hyperplasia, which features not only expansion of undifferentiated basal keratinocytes but morphologic and functional changes such as epithelial-mesenchymal transition (EMT), contributing to the inflammatory milieu as well as decreased epithelial barrier functions. In the stromal compartment of EoE, activation of myofibroblasts leads to fibrostenotic disease, the most serious functional consequence in EoE inflammation. These alterations in keratinocytes and fibroblasts have been hypothesized to create a unique epithelial-stromal cross-talk mediated by EoE-relevant cytokines. We have used OTC to investigate the role of transforming growth factor-β1, tumor necrosis factor-α, and interleukin-1β as essential mediators in EMT and myofibroblast activation in the context of EoE to corroborate their molecular profiling of endoscopic biopsy specimens from EoE patients.

The role of cytokines in EoE has been evaluated in a 3D organoid system in which transforming growth factor-β1, tumor necrosis factor-α, and interleukin-13 stimulated the expansion of basal cells in single–cell–derived murine esophageal 3D organoids, recapitulating reactive epithelial changes and basal cell hyperplasia as found in human EoE biopsy specimens. We envision the field moving toward optimized 3D organoid culture to allow expansion and passaging of human and murine organoids from single cells. Such protocols will allow for generation of organoids from patients with normal mucosa, GERD, and EoE, as well as correlation with human pathology found on biopsy specimens. Our group recently optimized 3D organoid culture conditions to allow expansion and multiple passages of both murine and human esophageal epithelial single–cell–derived organoids in a common and simplified culture medium (Kasagi et al, CMGH in press). In this study, human esophageal organoids were generated from biopsy specimens of patients with normal mucosa, GERD, and EoE. These 3D organoids showed hyperplasia in the presence of tumor necrosis factor-α or interleukin-13, indicating that reactive epithelial changes in situ may indeed occur in response to the inflammatory milieu.
Modeling Neoplastic and Preneoplastic Conditions in 3D

ESCC and EADC, 2 major esophageal cancers, and their precursor lesions have been modeled in 3D OTC while 3D organoid models of esophageal cancers are emerging (Table 1). Both OTC and 3D organoids permit morphologic assessment of a broad range of cancer cell characteristics, including proliferation (Ki67),59 DNA damage (γH2AX),129 epithelial-mesenchymal transition (E-cadherin and vimentin),50,59 and CSC markers (eg, CD44),116 in conjunction with genetic or pharmacologic modifications of cancer cells and fibroblasts. In OTC, pharmacologic treatment is not selective for either cancer cells or fibroblasts, although genetic modifications (eg, RNA interference or tetracycline- or tamoxifen-inducible systems) can be performed in a cell type–specific manner.49,53,54,59 Because cancer cells invade collectively or individually, cell invasion is evaluated quantitatively by measuring the area of invasive tumor islands within the stromal compartment.49,53,54,59 Laser capture microdissection can be used to analyze molecular changes in invasive cells.56,57 Moreover, conditioned media can be used for protein analyses to determine growth factors and cytokines that may influence cancer cell invasion and other activities.49

ESCC and Squamous Dysplasia

ESCC develops from squamous dysplasia as a common histologic precursor lesion.132,133 Studies on ESCC using 3D organoids are emerging, in which our group has established protocols to generate esophageal 3D organoids from murine ESCC and precancerous lesions.105 By using cell-lineage traceable transgenic mice, we have documented that ESCC cells arise from basal keratinocytes. Moreover, 3D organoids from conditional Notch1 knockout mice showed the role of Notch1 in EMT in premalignant and advanced ESCC lesions.105 In human ESCC patients, 3D organoids from diagnostic biopsy specimens may be used for molecular profiling as well as prediction of chemotherapy and radiation sensitivity, thus having a translational potential for personalized medicine.

OTC has served as a robust platform to study malignant transformation of esophageal keratinocytes and invasive disease progression of ESCC (Table 1). Multiple ESCC cell lines show invasive growth in OTC.49,52,108,124 Common genetic lesions in ESCC include inactivation of the p53, p120 catenin, and p16INK4A tumor-suppressor genes, as well as overexpression of the cyclin D1 and EGFR oncoproteins.134 Our genetically engineered mouse models of ESCC, which include targeting of EGFR and cyclin D1 to esophageal epithelium coupled with or without chemically induced carcinogenesis, show esophageal epithelial hyperplasia17 and dysplasia,134,135 respectively. ESCC develops in cyclin D1 transgenic mice upon p53 loss.136 These in vivo approaches now have been coupled with ex vivo 3D organoids and complemented with OTC in vitro.

EPC2-hTERT derivatives show hyperplasia (eg, EPC2-hTERT-EGFR-p53R175H53,59 and EPC2-p53R175H53,105,116) in OTC, depending on their genotypes. In particular, concurrent EGFR overexpression and p53 mutation appeared to be necessary for malignant transformation and invasive growth of human esophageal keratinocytes.53,136 Growing in a concentric manner, invasive tumor cell nests within the stromal compartment of OTC often show central cornification reminiscent of keratin pearl,53,56,58,59 a hallmark of well-differentiated ESCC. Similar keratinization is seen in ESCC 3D organoids.105

Laser capture microdissection in OTC and gene expression profiling showed unique molecular signatures at the onset of invasive growth of tumorigenic cells, but not premalignant nontumorigenic cells.56–58,137 Critical molecules identified and studied in OTC related to early neoplastic changes and ESCC cell invasion include markers for EMT, matrix metalloproteinase-9,53 periostin,56,113 p120 catenin,111 c-Met,49 IGFBP3,57 STAT1,113 Wnt10A,114 Notch,59,138 and activin A.135 In addition, OTC showed cancer cell heterogeneity with distinct growth and invasive characteristics defined by distinct CD44 isoforms, EMT, and antioxidant capacity.116,136 Moreover, fibroblasts were found to support ESCC cell invasion depending on fibroblast AKT activity75 as well as hepatocyte growth factor, the ligand for c-Met.49 Treatment of ESCC cells in OTC with bortezomib, a 26S proteasome inhibitor, induced apoptotic cancer cell death via concurrent activation of the p38 mitogen-activated protein kinase pathway.124

Barrett’s Esophagus and EADC

Barrett’s esophagus, intestinal metaplasia in the esophagus, has been linked to EADC development.135 Replacing normal squamous epithelium with columnar epithelium with mucin-filled goblet cells proposed theories regarding the BE cell origin include BE stem/progenitor cells residing at the squamous columnar junction, residual embryonic stem cells, transdifferentiation of esophageal keratinocytes, and esophageal glands.14

Recent studies using 3D culture systems have provided additional insights. Yamamoto et al140 induced goblet-like cells via the air–liquid interface with BE biopsy-derived colony-forming cells. Sato et al141 generated human BE tissue–derived organoids under conditions including pharmacologic Notch inhibition to promote secretory cell lineage differentiation whereas addition of fibroblast growth factor-10 permitted long-term passage of BE-derived organoids. Lee et al141 showed that cholecystokinin-2–receptor expressing cardiac progenitor cells may give rise to intestinal metaplasia and dysplasia in response to hypergastrinemia in murine cell–lineage tracing experiments coupled with organoid formation assays. Jiang et al142 identified unique p63-positive transitional basal cells expressing cytokeratins K5 and K7 as a putative BE cell of origin along with functional validation in 3D organoid assays. In addition, von Furstenberg et al143 used a porcine model of epithelial injury and human tissues to identify 2 distinct esophageal submucosal gland–derived cells expressing p63 or K7 that
give rise to squamous and ductal 3D spheroid structures, respectively.

BE-derived cell lines have been characterized in OTC where all-trans-retinoic acid was found to influence the phenotypic switch from squamous-like multilayered epithelial cells to columnar epithelial cells. In addition, inflammatory mediators such as interleukin-1β and COX-2 have been implicated in BE development. COX-2 over-expression resulted in the development of intestinal mucin-filled epithelia. OTC also has been used to study potential transdifferentiation of cell types. By using esophageal keratinocytes in OTC, Stairs et al found that transdifferentiation may be regulated by Cdx1 homeodomain transcription factor and the -Myc pathway, which cooperate to promote mucin production and changes in keratin expression, recapitulating gene expression profiles in BE. Moreover, genetic or pharmacologic inhibition of Notch appeared to facilitate transdifferentiation of esophageal keratinocytes toward columnar-like cell phenotypes in OTC with concurrent expression of Cdx1 and c-Myc. Wang et al have used the in vivo transplant culture system to show that hedgehog signaling promotes esophageal embryogenesis and BE development in the esophageal squamous epithelial cells in response to acid-mediated injury in which hedgehog activates transcription factor Forkhead Box A2 to induce intestinal mucin MUC2 and MUC2 processing protein AGR2. Aberrant hedgehog activation in keratinocytes leads to hyperproliferation in OTC, warranting investigating the role of hedgehog in esophageal OTC in the presence of exposure to acids.

To date, the 3D organoid has not been used to characterize EADC cells; however, OTC has been used to document mucin production and the invasiveness of several EADC cell lines (OE19, FLO-1, and MDF-1), in which the role of cancer-associated fibroblasts expressing periostin via the phosphatidylinositol 3-kinase was evaluated. Cancer-associated fibroblasts promote invasion via fibroblast-derived periostin. OTC served as a testing platform for molecularly targeted therapeutics including EGFR, mutant p53, and PIK3CA.

Conclusions and Future Perspectives

Esophageal 3D culture systems including OTC and the organoid system have provided substantial molecular and mechanistic insights into a number of physiological and pathologic conditions. Addition of immune cells and potentially other nonepithelial components in epithelial-fibroblast coculture–based OTC may open new avenues of research and preclinical drug testing. Single-cell–derived 3D esophageal organoids may provide sustainable resources for functional and mechanistic investigations into esophageal physiological and pathologic conditions with applications for both basic research and personalized medicine; however, further optimization may be required to permit 3D organoid growth from esophagi with normal and differential disease conditions (eg, ESCC and EADC). The necessity of differential culture conditions for different species, in particular human and murine esophageal 3D organoids, remains to be elucidated. It also needs to be determined how faithfully organoids mimic the genetics, epigenetics, and biology of the originating tissues after extended ex vivo culture. Developing an organoid bank of patient-derived esophageal 3D organoids may facilitate discovery and validation of novel translational applications in the setting of precision medicine, including testing chemotheraphy and radiation therapy in a moderate-to-high throughput manner for neoplastic organoids. Unlike neoplastic conditions, reactive epithelial changes in conditions such as GERD and EoE may be normalized ex vivo. Given disease susceptibility, polymorphic loci such as Aldh2 for esophageal carcinogenesis and Tslp for EoE, 3D esophageal organoids may be used to determine individual esophageal epithelial sensitivity to environmental toxic agents such as alcohol metabolites, tobacco smoke constituents, as well as disease-specific inflammatory cytokines. The influence of other environmental factors such as microbiota (bacteria, fungi, and viruses) and their interactions with antibiotics and probiotics upon epithelial biology may be tested using 3D esophageal organoids.

References

1. Doupe DP, Alcolea MP, Roshan A, Zhang G, Klein AM, Simons BD, Jones PH. A single progenitor population switches behavior to maintain and repair esophageal epithelium. Science 2012;337:1091–1093.
2. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human keratocytes: patterns of expression in normal epithelia, tumors and cultured cells. Cell 1982;31:11–24.
3. Grace MP, Kim KH, True LD, Fuchs E. Keratin expression in normal esophageal epithelium and squamous cell carcinoma of the esophagus. Cancer Res 1985;45:841–846.
4. Daniely Y, Liao G, Dixon D, Linnoila RI, Lori A, Randell SH, Oren M, Jetten AM. Critical role of p63 in the development of a normal esophageal and tracheobronchial epithelium. Am J Physiol Cell Physiol 2004;287:C171–C181.
5. Que J, Okubo T, Goldenring JR, Nam KT, Kurotani R, Morrissey EE, Taranova O, Pevny LH, Hogan BL. Multiple dose-dependent roles for Sox2 in the patterning and differentiation of anterior foregut endoderm. Development 2007;134:2521–2531.
6. Okumura T, Shimada Y, Imamura M, Yasumoto S, Neurotrophin receptor p75(NTR) characterizes human esophageal keratinocyte stem cells in vitro. Oncogene 2003;22:4017–4026.
7. Croagh D, Phillips WA, Redvers R, Thomas RJ, Kaur P. Identification of candidate murine esophageal stem cells using a combination of cell kinetic studies and cell surface markers. Stem Cells 2007;25:313–318.
8. Viaene AI, Baert JH. Expression of cytokeratin-mRNAs in squamous-cell carcinoma and balloon-cell formation of human oesophageal epithelium. Histochem J 1995;27:69–78.
9. Banks-Schlegel S, Green H. Involucrin synthesis and tissue assembly by keratinocytes in natural and cultured human epithelia. J Cell Biol 1981;90:732–737.
10. Dale BA, Scofield JA, Hennings H, Stanley JR, Yuspa SH. Identification of filagrin in cultured mouse keratinocytes and its regulation by calcium. J Invest Dermatol 1983;81:90s–95s.

11. Croagh D, Frede J, Jones PH, Kaur P, Partensky C, Phillips WA. Esophageal stem cells and genetics/epigenetics in esophageal cancer. Ann N Y Acad Sci 2014;1325:8–14.

12. DeWard AD, Cramer J, Lagasse E. Cellular heterogeneity in the mouse esophagus implicates the presence of a nonquiescent epithelial stem cell population. Cell Rep 2014;9:701–711.

13. Quante M, Abrams JA, Lee Y, Wang TC. Barrett esophagus: what a mouse model can teach us about human disease. Cell Cycle 2012;11:4328–4338.

14. Nakagawa H, Whelan K, Lynch JP. Mechanisms of Barrett’s oesophagus: intestinal differentiation, stem cells, and tissue models. Best Pract Res Clin Gastroenterol 2015;29:3–16.

15. Bailey T, Biddlestone L, Shepherd N, Barr H, Warner P, Jankowski J. Altered cadherin and catenin complexes in the Barrett’s esophagus-dysplasia-adenocarcinoma sequence: correlation with disease progression and dedifferentiation. Am J Pathol 1998;152:135–144.

16. Nair KS, Naidoo R, Chetty R. Expression of cell adhesion molecules in oesophageal carcinoma and its prognostic value. J Clin Pathol 2005;58:343–351.

17. Sherrill JD, Kc K, Wu D, Djukic Z, Caldwell JM, Stucke EM, Orlando RC, Hogan SP, Rothenberg ME. Desmoglein-1 regulates esophageal epithelial barrier function and immune responses in eosinophilic esophagitis. Mucosal Immunol 2014;7:718–729.

18. Stairs DB, Bayne LJ, Rhoades B, Vega ME, Waldron TJ, Kalabis J, Klein-Szanto A, Lee JS, Katz JP, Diehl JA, Reynolds AB, Vonderheide RH, Rustgi AK. Deletion of p120-catenin results in a tumor microenvironment with inflammation and cancer that establishes it as a tumor suppressor gene. Cancer Cell 2011;19:470–483.

19. Katayama M, Akaishi T, Nishihiro T, Kasai M, Kan M, Yamane I. Primary culture of human esophageal epithelial cells. Tohoku J Exp Med 1984;143:129–140.

20. Nishihiro T, Hashimoto Y, Katayama M, Mori S, Kuroki T. Molecular and cellular features of esophageal cancer cells. J Cancer Res Clin Oncol 1993;119:441–449.

21. Stenn KS, Stenn JO. Organ culture of adult esophageal mucosa in a defined medium. J Invest Dermatol 1976;66:302–305.

22. Hopwood D, Milne G, Jankowski J, Howat K, Johnston D, Wormsley KG. Secretory and absorptive activity of oesophageal epithelium: evidence of circulating mucosubstances. Histochem J 1994;26:41–49.

23. Hopwood D, Spiers EM, Ross PE, Anderson JT, McCullough JB, Murray FE. Endocytosis of fluorescent microspheres by human oesophageal epithelial cells: comparison between normal and inflamed tissue. Gut 1995;37:598–602.

24. Arsenault P, Menard D. Autoradiographic localization of [3H]-thymidine incorporation in developing human esophagus. Anat Rec 1988;220:313–317.

25. Menard D, Arsenault P. Maturation of human fetal esophagus maintained in organ culture. Anat Rec 1987;217:348–354.

26. Rieder F, Cheng L, Barnett KM, Chak A, Cooper GS, Isenberg G, Ray M, Katz JA, Catanzaro A, O’Shea R, Post AB, Wong R, Sivak MV, McCormick T, Phillips M, West GA, Willis JE, Blancani P, Fiocchi C. Gastro-esophageal reflux disease-associated esophagitis induces endogenous cytokine production leading to motor abnormalities. Gastroenterology 2007;132:154–165.

27. Chang CL, Lao-Sirieix P, Save V, De La Cueva Mendez G, Laskey R, Fitzgerald RC. Retinoic acid-induced glandular differentiation of the esophagus. Gut 2007;56:906–917.

28. Kaur BS, Ouatu-Lascar R, Omary MB, Triadafilopoulos G. Bile salts induce or blunt cell proliferation in Barrett’s esophagus in an acid-dependent fashion. Am J Physiol Gastrointest Liver Physiol 2000;278:G1000–G1009.

29. Shirvani VN, Ouatu-Lascar R, Kaur BS, Omary MB, Triadafilopoulos G. Cyclooxygenase 2 expression in Barrett’s esophagus and adenocarcinoma: an ex vivo induction by bile salts and acid exposure. Gastroenterology 2000;118:487–496.

30. Fitzgerald RC, Omary MB, Triadafilopoulos G. Dynamic effects of acid on Barrett’s esophagus. An ex vivo proliferation and differentiation model. J Clin Invest 1996;98:2120–2128.

31. Fitzgerald RC, Omary MB, Triadafilopoulos G. Altered sodium-hydrogen exchange activity is a mechanism for acid-induced hyperproliferation in Barrett’s esophagus. Am J Physiol 1998;275:G47–G55.

32. Mothersill C, Cusack A, MacDonnell M, Hennessy TP, Seymour CB. Differential response of normal and tumour oesophageal explants cultures to radiation. Acta Oncol 1988;27:275–280.

33. Hillman EA, Vocci MJ, Schurch W, Harris CC, Trump BF. Human esophageal organ culture studies. Methods Cell Biol 1980;21B:331–348.

34. Vocci MJ, Combs JW, Hillman EA, Resau JH, Trump BF. The cell kinetics of the adaptation of the human esophagus to organ culture. In Vitro 1983;19:881–891.

35. Kondo Y, Young GP, Rose I, Whitehead RH. Organ specificity of epithelial cells grown in tissue culture from explants obtained from various levels of the rat gut. Exp Cell Res 1985;159:158–170.

36. Bell E, Ivarsson B, Merrill C. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. Proc Natl Acad Sci U S A 1979;76:1274–1278.

37. Bell E, Ehrlich HP, Buttle DJ, Nakatsui T. Living tissue formed in vitro and accepted as skin-equivalent tissue of full thickness. Science 1981;211:1052–1054.

38. Bell E, Sher S, Hull B, Merrill C, Rosen S, Chanson A, Asselineau D, Dubertret L, Coulomb B, Lapiere C, Nusgens B, Neveux Y. The reconstitution of living skin. J Invest Dermatol 1983;81:2s–10s.
39. Prunieras M, Regnier M, Woodley D. Methods for cultivation of keratinocytes with an air-liquid interface. J Invest Dermatol 1983;81:228–33.

40. Asselineau D, Prunieras M. Reconstruction of ‘simplified’ skin: control of fabrication. Br J Dermatol 1984;111(Suppl 27):219–222.

41. Ohashi S, Natsuizaka M, Yashiro-Ohtani Y, Kalman RA, Nakagawa M, Wu L, Klein-Szanto AJ, Herlyn M, Diehl JA, Katz JP, Pear WS, Seykora JT, Nakagawa H. NOTCH1 and NOTCH3 coordinate esophageal squamous differentiation through a CSL-dependent transcriptional network. Gastroenterology 2010;139:2113–2123.

42. Tsunenaga M, Kohno Y, Horii I, Yasumoto S, Huh NH, Tachikawa T, Yoshiki S, Kuroki T. Growth and differentiation properties of normal and transformed human keratinocytes in organotypic culture. Jpn J Cancer Res 1994;85:238–244.

43. McCance DJ, Kopan R, Fuchs E, Laimins LA. Human papillomavirus type 16 alters human epithelial cell differentiation in vitro. Proc Natl Acad Sci U S A 1988;85:7169–7173.

44. Hudson JB, Bedell MA, McCance DJ, Laimins LA. Immortalization and altered differentiation of human keratinocytes in vitro by the E6 and E7 open reading frames of human papillomavirus type 18. J Virol 1990;64:519–526.

45. Blanton RA, Perez-Reyes N, Merrick DT, McDougall JK. Epithelial cells immortalized by human papillomaviruses have premalignant characteristics in organotypic culture. Am J Pathol 1991;138:673–685.

46. Merrick DT, Blanton RA, Gown AM, McDougall JK. Altered expression of proliferation and differentiation markers in human papillomavirus 16 and 18 immortalized epithelial cells grown in organotypic culture. Am J Pathol 1992;140:167–177.

47. Andl CD, Mizushima T, Nakagawa H, Oyama K, Harada H, Chruma K, Herlyn M, Rustgi AK. Epidermal growth factor receptor mediates increased cell proliferation, migration, and aggregation in esophageal keratinocytes in vitro and in vivo. J Biol Chem 2003;278:1824–1830.

48. Harada H, Nakagawa H, Oyama K, Takaoka M, Andl CD, Jacobmeier B, von Werder A, Enders GH, Opitz OG, Rustgi AK. Telomerase induces immortalization of human esophageal keratinocytes without p16INK4a inactivation. Mol Cancer Res 2003;1:729–738.

49. Grugan KD, Miller CG, Yao Y, Michaylira CZ, Ohashi S, Klein-Szanto AJ, Diehl JA, Herlyn M, Han M, Nakagawa H, Rustgi AK. Fibroblast-secreted hepatocyte growth factor plays a functional role in esophageal squamous cell carcinoma invasion. Proc Natl Acad Sci U S A 2010;107:11026–11031.

50. Underwood TJ, Derouet MF, White MJ, Noble F, Moutasim KA, Smith E, Drew PA, Thomas GJ, Primrose JN, Blaydes JP. A comparison of primary esophageal squamous epithelial cells with HET-1A in organotypic culture. Bioi Cell 2010;102:635–644.

51. Underwood TJ, Hayden AL, Derouet M, Garcia E, Noble F, White MJ, Thirdborough S, Mead A, Clemons N, Mellone M, Uzoh C, Primrose JN, Blaydes JP, Thomas GJ. Cancer-associated fibroblasts predict poor outcome and promote periostrin-dependent invasion in esophageal adenocarcinoma. J Pathol 2015;235:466–477.

52. Kalabis J, Wong GS, Vega ME, Natsuizaka M, Robertson ES, Herlyn M, Nakagawa H, Rustgi AK. Isolation and characterization of mouse and human esophageal epithelial cells in 3D organotypic culture. Nat Protoc 2012;7:235–246.

53. Okawa T, Michaylira CZ, Kalabis J, Stairs DB, Nakagawa H, Andl CD, Johnstone CN, Klein-Szanto AJ, El-Deiry WS, Cukierman E, Herlyn M, Rustgi AK. The functional interplay between EGFR overexpression, hTERT activation, and p53 mutation in esophageal epithelial cells with activation of stromal fibroblasts induces tumor development, invasion, and differentiation. Genes Dev 2007;21:2788–2803.

54. Oyama K, Okawa T, Nakagawa H, Takaoka M, Andl CD, Kim SH, Klein-Szanto A, Diehl JA, Herlyn M, El-Deiry W, Rustgi AK. AKT induces senescence in primary esophageal epithelial cells but is permissive for differentiation as revealed in organotypic culture. Oncogene 2007;26:2353–2364.

55. Stairs DB, Nakagawa H, Klein-Szanto A, Mitchell SD, Silberg DG, Tobias JW, Lynch JP, Rustgi AK. Cdx1 and c-Myc foster the initiation of transdifferentiation of the normal esophageal squamous epithelium toward Barrett’s esophagus. PLoS One 2008;3:e3534.

56. Michaylira CZ, Wong GS, Miller CG, Gutierrez CM, Nakagawa H, Hammond R, Klein-Szanto AJ, Lee JS, Kim SB, Herlyn M, Diehl JA, Gimotty P, Rustgi AK. Periostin, a cell adhesion molecule, facilitates invasion in the tumor microenvironment and annotates a novel tumor-invasive signature in esophageal cancer. Cancer Res 2010;70:5281–5292.

57. Natsuizaka M, Ohashi S, Wong GS, Ahmadi A, Kalman RA, Budo D, Klein-Szanto AJ, Herlyn M, Diehl JA, Nakagawa H. Insulin-like growth factor-binding protein-3 promotes transforming growth factor-{beta}1-mediated epithelial-to-mesenchymal transition and motility in transformed human esophageal cells. Carcinogenesis 2010;31:1344–1353.

58. Ohashi S, Natsuizaka M, Naganuma S, Kagawa S, Kinura S, Itoh H, Kalman RA, Nakagawa M, Darling DS, Basu D, Gimotty PA, Klein-Szanto AJ, Diehl JA, Herlyn M, Nakagawa H. A NOTCH3-mediated squamous cell differentiation program limits expansion of EMT-competent cells that express the ZEB transcription factors. Cancer Res 2011;71:6836–6847.

59. Naganuma S, Whelan KA, Natsuizaka M, Kagawa S, Kinugasa H, Chang S, Subramanian H, Rhoades B, Ohashi S, Itoh H, Herlyn M, Diehl JA, Gimotty PA, Klein-Szanto AJ, Nakagawa H. Notch receptor inhibition reveals the importance of cyclin D1 and Wnt signaling in invasive esophageal squamous cell carcinoma. Am J Cancer Res 2012;2:459–475.

60. Vega ME, Giroux V, Natsuizaka M, Liu M, Klein-Szanto AJ, Stairs DB, Nakagawa H, Wang KK, Wang TC, Lynch JP, Rustgi AK. Inhibition of Notch signaling enhances transdifferentiation of the esophageal squamous
epithelium towards a Barrett’s-like metaplasia via KLF4. Cell Cycle 2014;13:3857–3866.

61. Croagh D, Cheng S, Tikoo A, Nandurkar S, Thomas RJ, Kaur P, Phillips WA. Reconstitution of stratified murine and human oesophageal epithelia in an in vivo transplant culture system. Scand J Gastroenterol 2008; 43:1158–1168.

62. Wang DH, Tiwari A, Kim ME, Clemons NJ, Regmi NL, Hodges WA, Berman DM, Montgomery EA, Watkins DN, Zhang X, Zhang Q, Jie C, Spechler SJ, Souza RF. Hedgehog signaling regulates FOXA2 in esophageal embryogenesis and Barrett’s metaplasia. J Clin Invest 2014;124:3767–3780.

63. Wang DH, Clemons NJ, Miyashita T, Dupuy AJ, Zhang W, Szczepnica A, Corcoran-Schwart IM, Wilburn DL, Montgomery EA, Wang JS, Jenkins NA, Copeland NA, Harmon JW, Phillips WA, Watkins DN. Aberrant epithelial-mesenchymal Hedgehog signaling characterizes Barrett’s metaplasia. Gastroenterology 2010;138:1810–1822.

64. Moscona A. Rotation-mediated histogenetic aggregation of dissociated cells. A quantifiable approach to cell interactions in vitro. Exp Cell Res 1961;22:455–475.

65. Moscona A, Moscona H. The dissociation and aggregation of cells from organ rudiments of the early chick embryo. J Anat 1952;86:287–301.

66. Weiss P, Taylor AC. Reconstitution of complete organs from single-cell suspensions of chick embryos in advanced stages of differentiation. Proc Natl Acad Sci U S A 1980;46:1177–1185.

67. Dangles-Marie V, Validire P, Wertheimer M, Richon S, Bovin C, Zeliszewski D, Vallancien G, Bellet D, Bellet D. Impact of human bladder cancer cell architecture on autologous T-lymphocyte activation. Int J Cancer 2002;98:51–56.

68. Dangles-Marie V, Richon S, El-Behi M, Echchakir H, Dorothee G, Thiery J, Validire P, Vergnon I, Menez J, Ladjimi M, Chouaib S, Bellet D, Mami-Chouaib F. A three-dimensional tumor cell defect in activating autologous CTLs is associated with inefficient antigen presentation correlated with heat shock protein-70 down-regulation. Cancer Res 2003;63:3682–3687.

69. Timmins NE, Dietmair S, Nielsen LK. Hanging-drop multicellular spheroids as a model of tumour angiogenesis. Angiogenesis 2004;7:97–103.

70. Korff T, Augustin HG. Integration of endothelial cells in multicellular spheroids prevents apoptosis and induces differentiation. J Cell Biol 1998;143:1341–1352.

71. Sarbia M, Bosing N, Hildebrandt B, Koldovsky P, Gerharz CD, Gabbert HE. Characterization of two newly established cell lines derived from squamous cell carcinomas of the oesophagus. Anticancer Res 1997;17:2185–2192.

72. Li ML, Aghjely J, Farson DA, Hatier C, Hassell J, Bissell MJ. Influence of a reconstituted basement membrane and its components on casein gene expression and secretion in mouse mammary epithelial cells. Proc Natl Acad Sci U S A 1987;84:136–140.

73. Andl CD, Fargnoli BB, Okawa T, Bowser M, Takaoka M, Nakagawa H, Klein-Szanto A, Hua X, Herlyn M, Rustgi AK. Coordinated functions of E-cadherin and transforming growth factor beta receptor II in vitro and in vivo. Cancer Res 2006;66:9878–9885.

74. Le Bras GF, Loomans HA, Taylor CJ, Revetta FL, Andl CD. Activin A balance regulates epithelial invasiveness and tumorigenesis. Lab Invest 2014;94:1134–1146.

75. Pastrana E, Silva-Vargas V, Doetsch F. Eyes wide open: a critical review of sphere-formation as an assay for stem cells. Cell Stem Cell 2011;8:486–498.

76. Weiswald LB, Bellet D, Dangles-Marie V. Spherical cancer models in tumor biology. Neoplasia 2015;17:1–15.

77. Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 1992;255:1707–1710.

78. Tsai ST, Wang PJ, Liu NJ, Lin PS, Chen CH, Chang WC. ICAM1 is a potential cancer stem cell marker of esophageal squamous cell carcinoma. PLoS One 2015;10:e0142834.

79. Zhao R, Quaroni L, Casson AG. Identification and characterization of stemlike cells in human esophageal adenocarcinoma and normal epithelial cell lines. J Thorac Cardiovasc Surg 2012;144:1192–1199.

80. Wang JL, Yu JP, Sun ZQ, Sun SP. Radiobiological characteristics of cancer stem cells from esophageal cancer cell lines. World J Gastroenterol 2014; 20:18296–18305.

81. Tang KH, Dai YD, Tong M, Chan YP, Kwan FS, Fu L, Qin YR, Tsao SW, Lung HL, Lung ML, Tong DK, Law S, Chan KW, Ma S, Guan XY. A CD90(+) tumor-initiating cell population with an aggressive signature and metastatic capacity in esophageal cancer. Cancer Res 2013;73:2322–2332.

82. Almanaa TN, Geusz ME, Jamasbi RJ. A new method for identifying stem-like cells in esophageal cancer cell lines. J Cancer 2013;4:536–548.

83. Zhang G, Ma L, Xie YK, Miao XB, Jin C. Esophageal cancer tumorspheres involve cancer stem-like populations with elevated aldehyde dehydrogenase enzymatic activity. Mol Med Rep 2012;6:519–524.

84. Song S, Ajani JA, Honjo S, Maru DM, Chen Q, Scott AW, Heallen TR, Xiao L, Hofstetter WL, Weston B, Lee JH, Wadhwa R, Sudo K, Stroehlein JR, Martin JF, Hung MC, Johnson RL. Hippo coactivator YAP1 upregulates SOX9 and endows esophageal cancer cells with stem-like properties. Cancer Res 2014;74:4170–4182.

85. Yue D, Zhang Z, Li J, Chen X, Ping Y, Liu S, Shi X, Li L, Wang L, Huang L, Zhang B, Sun Y, Zhang Y. Transforming growth factor-beta1 promotes the migration and invasion of sphere-forming stem-like cell subpopulations in esophageal cancer. Exp Cell Res 2015;336:141–149.

86. Kano Y, Konno M, Ohta K, Haraguchi N, Nishikawa S, Kageya Y, Hamabe A, Hasegawa S, Ogawa H, Fukusumi T, Noguchi Y, Ozaki M, Kudo T, Sakai D, Sato T, Ishii M, Mizohata E, Inoue T, Mori M, Doki Y, Ishii H. Jumonji/Arid1b (Jarid1b) protein modulates human esophageal cancer cell growth. Mol Clin Oncol 2013;1:753–757.

87. Honjo S, Ajani JA, Scott AW, Chen Q, Skinner HD, Stroehlein J, Johnson RL, Song S. Metformin sensitizes chemotherapy by targeting cancer stem cells and the
mTOR pathway in esophageal cancer. Int J Oncol 2014; 45:567–574.

88. Wang Z, Da Silva TG, Jin K, Han X, Ranganathan P, Zhu X, Sanchez-Mejias A, Bai F, Li B, Fei DL, Weaver K, Carpio RV, Moscwitz AE, Koshenkov VP, Sanchez L, Sparling L, Pei XH, Franceschini D, Ribeiro A, Robbins DJ, Livingstone AS, Capobianco AJ. Notch signaling drives stemness and tumorigenicity of esophageal adenocarcinoma. Cancer Res 2014;74:6364–6374.

89. Almanna TN, Geusz ME, Jamasbi RJ. Effects of curcumin on stem-like cells in human esophageal squamous carcinoma cell lines. BMC Complement Altern Med 2012; 12:195.

90. Xu DD, Zhou PJ, Wang Y, Zhang L, Fu WY, Ruan BB, Xu HP, Hu CZ, Tian L, Qin JH, Wang S, Wang X, Li YC, Liu QY, Ren Z, Zhang R, Wang YF. Reciprocal activation between STAT3 and miR-181b regulates the proliferation of esophageal cancer stem-like cells via the CYLD pathway. Mol Cancer 2016;15:40.

91. Li B, Xu WW, Han L, Chan KT, Tsao SW, Lee NPY, Law S, Xu LY, Li EM, Chan KW, Qin YR, Guan XY, He QY, Chen ALM. MicroRNA-377 suppresses initiation and progression of esophageal cancer by inhibiting CD133 and VEGF. Oncogene 2017;36:3968–4000.

92. Zhao JS, Li WJ, Ge D, Zhang PJ, Li JJ, Lu CL, Ji XD, Guan DX, Gao H, Xu LY, Li EM, Soukiasian H, Koeffler HP, Wang XF, Xie D. Tumor initiating cells in esophageal squamous cell carcinomas express high levels of CD44. PLoS One 2011;6:e21419.

93. Sato T, Vries RG, Snippef HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, Clevers H. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009;459:262–265.

94. Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, Van Houdt WJ, Pronk A, Van Gorp J, Siersema PD, Clevers H. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett’s epithelium. Gastroenterology 2011;141:1762–1772.

95. van de Wetering M, Francies HE, Francis JM, Bounova G, Iorio F, Pronk A, van Houdt W, van Gorp J, Taylor-Weiner A, Kester L, McLaren-Douglas A, Blokker J, Jaksani S, Bartfeld S, Volckman R, van Sluis P, Li VS, Seepo S, Sekhar Pedamallu C, Cibulsikis K, Carter SL, McKenna A, Lawrence MS, Lichtenstein L, Stewart C, Koster J, Versteeg R, van Oudenaarden A, Sae-z Rodriguez J, Vries RG, Getz G, Wessels L, Stratton MR, McDermott U, Meyerson M, Garnett MJ, Clevers H. Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell 2015;161:933–945.

96. Matano M, Date S, Shimokawa M, Takano A, Fuji M, Ohta Y, Watanabe T, Kanai T, Sato T. Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. Nat Med 2015;21:256–262.

97. Sato T, Clevers H. SnapShot: growing organoids from stem cells. Cell 2015;161:1700–e11.

98. Dutta D, Heo I, Clevers H. Disease modeling in stem cell-derived 3D organoid systems. Trends Mol Med 2017; 23:393–410.

99. Weeber F, van de Wetering M, Hoogstraat M, Dijkstra KK, Krijgsman O, Kuijman T, Gadella-van Hooijdonk CG, van der Velden DL, Peeper DS, Cuppen EP, Vries RG, Clevers H, Voest EE. Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases. Proc Natl Acad Sci U S A 2015;112:13308–13311.

100. Schutte M, Risch T, Abdavi-Azar N, Boehnke K, Schumacher D, Keil M, Ylidiriman R, Jandrasits C, Borodina T, Amstislavskiy V, Worth CL, Schweiger C, Liebs S, Lange M, Warnatz HJ, Butcher LM, Barrett JE, Sultan M, Wierling C, Golob-Schwarzl N, Lax S, Uranitsch S, Becker M, Welte Y, Regan JL, Silvestrov M, Kehler I, Fusi A, Kessler T, Herwig R, Landegren U, Wiencek D, Nilsson M, Velasco JA, Garin-Chesa P, Reinhard C, Beck S, Schafer R, Regenbrecht CR, Henderson D, Lange B, Haybaeeck J, Keilholz U, Hoffmann J, Lehrhac H, Yaso ML. Molecular dissection of colorectal cancer in pre-clinical models identifies biomarkers predicting sensitivity to EGFR inhibitors. Nat Commun 2017;8:14262.

101. Boj SF, Hwang CI, Baker LA, Engle DD, Corbo V, Jager M, Ponz-Sarvise M, Tiriac H, Spector MS, Gracanin A, Oni T, Yu KH, van Bokel R, Huch M, Rivera KD, Wilson JP, Feigin ME, Ohlund D, Handly-Santana A, Ardito-Abraham CM, Ludwig M, Elyada E, Alagesan B, Biffi G, Yordanov GN, Delucze B, Creighton B, Wright K, Park Y, Morsink FH, Molenaa IQ, Borel Rinkes IH, Cuppen E, Hao Y, Jin Y, Nijman IJ, Iacobuzio-Danahue C, Leach SD, Pappin DJ, Hammell M, Klimstra DS, Basturk O, Hubran RH, Offerhaus GJ, Vries RG, Clevers H, Tuveson DA. Organoid models of human and mouse ductal pancreatic cancer. Cell 2015;160:324–338.

102. Huang L, Holtzinger A, Jagan I, BeGora M, Lohse I, Ngai N, Nostro C, Wang R, Muthuswamy LB, Crawford HC, Arrowsmith C, Kalloger SE, Renouf DJ, Connor AA, Cleary S, Schaeffer DF, Roehl R, Tsao MS, Gallinger S, Keller G, Muthuswamy SK. Ductal pancreatic cancer modeling and drug screening using human pluripotent stem cell- and patient-derived tumor organoids. Nat Med 2015;21:1364–1371.

103. Gao D, Vela I, Stbner A, laquitoa PJ, Karthaus WR, Gopalan A, Dowling C, Wanjala JN, Undvall EA, Arora VK, Wongsipat J, Kossai M, Ramazanoglu S, Barboza LP, Di W, Cao Z, Zhanh QF, Sirato I, Ran L, Donaldson T, Beltran H, Mosquera JM, Touijer KA, Scardino PT, Laudone VP, Curtis KR, Rathkopf DE, Morris MJ, Danila DC, Slovin SF, Solomon SB, Eastham JA, Chi P, Carver B, Rubin MA, Schier H, Clevers H, Sawyer LS, Chen Y. Organoid cultures derived from patients with advanced prostate cancer. Cell 2014;159:176–187.

104. Paul C, Hopkins BD, Prandi D, Shaw R, Fedrizzi T, Stbner A, Sailer V, Augello M, Puca L, Rosati R, McNay TJ, Churakova Y, Cheung C, Triscott J, Pisapia D, Rao R, Mosquera JM, Robinson B, Faltas BM, Emerling B, Gadi VK, Bernard B, Elemento O, Beltran H, Demichelis F, Kemp C, Grandori C, Cantley LC, Rubin MA. Personalized in vitro and in vivo cancer models to guide precision medicine. Cancer Discov 2017;7:462–477.
105. Natsuizaka M, Whelan KA, Hamilton KE, Hamil
106. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
107. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
108. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
109. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
110. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
111. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
112. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
113. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
114. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
115. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
116. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
117. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
118. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
119. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
120. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
121. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
122. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
123. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
124. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
125. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
126. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
127. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
128. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
129. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
130. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
131. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
132. Natsuizaka M, Whelan KA, Kagawa S, Tanaka K,
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Masterson JC, Fernando SD, Godwin BC, Klein-Szanto AJ, Chikwava K, Ruchelli ED, Hamilton KE, Muir AB, Wang ML, Furuta GT, Falk GW, Spergel JM, Nakagawa H. Autophagy mediates epithelial cytoprotection in eosinophilic oesophagitis. Gut 2017; 66:1197–1207.

Okano J, Gaslightwala I, Birnbaum MJ, Rustgi AK, Nakagawa H. Akt/protein kinase B isoforms are differentially regulated by epidermal growth factor stimulation. J Biol Chem 2000;275:30934–30942.

Yan YX, Nakagawa H, Lee MH, Rustgi AK. Transforming growth factor-alpha enhances cyclin D1 transcription through the binding of early growth response protein to a cis-regulatory element in the cyclin D1 promoter. J Biol Chem 1997;272:33181–33190.

Tanaka K, Whelan KA, Chandramouleeswaran PM, Kagawa S, Rustgi SL, Noguchi C, Guha M, Srinivasan S, Amanuma Y, Ohashi S, Muto M, Klein-Szanto AJ, Noguchi E, Avadhani NG, Nakagawa H. ALDH2 modulates autophagy flux to regulate acetaldehyde-mediated toxicity thresholds. Am J Cancer Res 2016;6:781–796.

Kasagi Y, Chandramouleeswaran PM, Whelan KA, Tanaka K, Giroux V, Sharma M, Wang J, Benitez AJ, DeMarshall M, Tobias JW, Hamilton KE, Falk GW, Spergel JM, Klein-Klein-Szanto AJ, Rustgi AK, Muir AB, Nakagawa H. The esophageal organoid system reveals functional interplay between notch and cytokines in reactive epithelial changes. Cell Molec Gastroenterol Hepatol 2018;5:333–352.

Kagalwalla FA, Akhtar N, Woodruff SA, Rea BA, Masterson JC, Mukkada V, Parashesse KR, Du J, Fillon S, Protheroe CA, Lee JJ, Amsden K, Melin-Aldana H, Capocelli KE, Furuta GT, Ackerman SJ. Eosinophilic esophagitis: epithelial mesenchymal transition contributes to esophageal remodeling and reverses with treatment. J Allergy Clin Immunol 2012;129:1387–1396 e7.

Wang GQ, Abnet CC, Shen Q, Lewin KJ, Sun XD, Roth MJ, Qiao YL, Mark SD, Dong ZW, Taylor PR, Dawsey SM. Histological precursors of oesophageal squamous cell carcinoma: results from a 13 year prospective follow up study in a high risk population. Gut 2005;54:187–192.

Nakagawa H, Katsu K, Rustgi AK. Biology of esophageal cancer. In: Rustgi AK, ed. Gastrointestinal Cancers. London: Elsevier, 2003:241–251.

Nakagawa H, Wang TC, Zukerberg L, Odze R, Togawa K, May GH, Wilson J, Rustgi AK. The targeting of the cyclin D1 oncoprote by an Epstein-Barr virus promoter in transgenic mice causes dysplasia in the tongue, esophagus and forestomach. Oncogene 1997;14:1185–1190.

Mueller A, Odze R, Jenkins TD, Shahesfaei A, Nakagawa H, Inomoto T, Rustgi AK. A transgenic mouse model with cyclin D1 overexpression results in cell cycle, epidermal growth factor receptor, and p53 abnormalities. Cancer Res 1997;57:5542–5549.

Opitz OG, Harada H, Suliman Y, Rhoades B, Sharpless NE, Kent R, Kopelovich L, Nakagawa H, Rustgi AK. A mouse model of human oral-esophageal cancer. J Clin Invest 2002;110:761–769.

Ohashi S, Natsuizaka M, Wong GS, Michaylira CZ, Grugan KD, Stairs DB, Kalabis J, Vega ME, Kalman RA, Nakagawa M, Klein-Szanto AJ, Herlyn M, Diehl JA, Rustgi AK, Nakagawa H. Epidermal growth factor receptor and mutant p53 expand an esophageal cellular subpopulation capable of epithelial-to-mesenchymal transition through ZEB transcription factors. Cancer Res 2010;70:4174–4184.

Kingusa H, Whelan KA, Tanaka K, Natsuizaka M, Long A, Guo A, Chang S, Kagawa S, Srinivasan S, Guha M, Yamamoto K, St Clair DK, Avadhani NG, Diehl JA, Nakagawa H. Mitochondrial SOD2 regulates epithelial-mesenchymal transition and cell populations defined by differential CD44 expression. Oncogene 2015;34:5229–5239.

Falk GW, Jacobson BC, Riddell RH, Rubenstein JH, El-Zimaity H, Drewes AM, Roark KS, Sontag SJ, Schnell TG, Leya J, Chejfec G, Richter JE, Jenkins G, Goldman A, Dvorak K, Nardone G. Barrett’s esophagus: prevalence-incidence and etiology-origins. Ann N Y Acad Sci 2011;1232:1–17.

Yamamoto Y, Wang X, Bertrand D, Kern F, Zhang T, Duleba M, Srivastava S, Khor CC, Hu Y, Wilson LH, Blazsky H, Rolshud D, Teh M, Liu J, Howitt BE, Vincent M, Crum CP, Nagarajan N, Ho KY, McKeon F, Xian W. Mutational spectrum of Barrett’s stem cells suggests paths to initiation of a precancerous lesion. Nat Commun 2016;7:10380.

Lee Y, Urbanska AM, Hayakawa Y, Wang H, Au AS, Luna AM, Chang W, Jin G, Bhagat G, Abrams JA, Friedman RA, Varro A, Wang KK, Boyce M, Rustgi AK, Sepulveda AR, Quante M, Wang TC. Gastrin stimulates a cholecystokinin-2-receptor-expressing cardia progenitor cell and promotes progression of Barrett’s-like esophagus. Oncotarget 2017;8:203–214.

Jiang M, Li H, Zhang Y, Yang Y, Lu R, Liu K, Lin S, Lan X, Wang H, Wu H, Zhu J, Zhou Z, Xu J, Lee DJ, Zhang L, Lee YC, Yuan J, Abrams JA, Wang TC, Sepulveda AR, Wu Q, Chen H, Sun X, She J, Chen X, Que J. Transitional basal cells at the squamous-columnar junction generate Barrett’s esophagus. Nature 2017;550:529–533.

von Furstenberg RJ, Li J, Stolarchuk C, Feder R, Campbell A, Kruger L, Gonzalez LM, Blikslasher AT, Cardona DM, McCall SJ, Henning SJ, Garman KS. Porcine esophageal submucosal gland culture model shows capacity for proliferation and differentiation. Cell Mol Gastroenterol Hepatol 2017;4:385–404.

Brellier F, Bergoglio V, Valin A, Barnay S, Chevallier-Lagente O, Vielh P, Spatz A, Gorry P, Avril MF, Magnaldo T. Heterozygous mutations in the tumor suppressor gene PATCHED provoke basal cell carcinoma-like features in human organotypic skin cultures. Oncogene 2008;27:6601–6606.

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Hiroshi Nakagawa conceived the manuscript; and Amanda B. Muir, Hiroshi Nakagawa, and Kelly A. Whelan wrote and edited the manuscript.

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The authors disclose no conflicts.

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