Published in final edited form as:

Dig Dis Sci. 2018 August ; 63(8): 2005–2012. doi:10.1007/s10620-018-5069-5.

Origins of Metaplasia in Barrett's Esophagus: Is this an Esophageal Stem or Progenitor Cell Disease?

Wei Zhang¹, David H. Wang^{1,2}

¹Esophageal Diseases Center, Department of Internal Medicine and the Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, Texas, USA

²Medical Service, Dallas VA Medical Center, Dallas, Texas, USA

Abstract

The incidence of esophageal adenocarcinoma has been increasing in Western countries over the past several decades. Though Barrett's esophagus, in which the normal esophageal squamous epithelium is replaced with metaplastic intestinalized columnar cells due to chronic damage from gastroesophageal reflux, is accepted as the requisite precursor lesion for esophageal adenocarcinoma, the Barrett's esophagus cell of origin and the molecular mechanism underlying esophageal epithelial metaplasia remain controversial. Much effort has been dedicated towards identifying the Barrett's esophagus cell of origin since this could lead to more effective prevention and treatment strategies for both Barrett's esophagus and esophageal adenocarcinoma. Previously, it was hypothesized that terminally differentiated esophageal squamous cells might undergo direct conversion into specialized intestinal columnar cells via the process of transdifferentiation. However, there is increasing evidence that stem and/or progenitor cells are molecularly reprogrammed through the process of transcommitment to differentiate into the columnar cell lineages that characterize Barrett's esophagus. Given that Barrett's esophagus originates at the gastroesophageal junction, the boundary between the distal esophagus and gastric cardia, potential sources of these stem and/or progenitor cells include columnar cells from the squamocolumnar junction or neighboring gastric cardia, native esophageal squamous cells, native esophageal cuboidal or columnar cells from submucosal glands or ducts, or circulating bone marrow-derived cells. In this review, we focus on native esophageal specific stem and/or progenitor cells and detail molecular mediators of transcommitment based on recent insights gained from novel mouse models and clinical observations from patients.

Keywords

Barrett's esophagus; transcommitment; transdifferentiation; squamous progenitor cell; squamous stem cell

Address correspondence to: David H. Wang, MD, PhD, Division of Hematology-Oncology, Department of Internal Medicine, UT Southwestern Medical Center, 5323 Harry Hines Blvd, MC 8584, Dallas, TX 75390-8584, Phone: 214-648-4180, Fax: 214-462-4953, David1.Wang@UTSouthwestern.edu.

Author Contributions: Drafting of the manuscript (WZ and DHW), critical revision of the manuscript for intellectual content (WZ and DHW), obtained funding (DHW).

Disclosures: The authors declare that they have no conflict of interest.

Over the last several decades, the incidence of esophageal adenocarcinoma has increased rapidly in Western countries with gastroesophageal reflux disease (GERD), Barrett's esophagus, and obesity as its major risk factors (1, 2). Surprisingly, this dramatic rise in the incidence of esophageal adenocarcinoma has occurred despite the increased utilization of a variety of powerful acid-suppressing medications to treat gastroesophageal reflux, highlighting the importance of developing alternative chemoprevention strategies (1). The precursor lesion for esophageal adenocarcinoma is Barrett's esophagus, the metaplastic change of the squamous epithelium lining the distal esophagus into a columnar epithelium forming glands, which can occur as a complication of GERD (2). In the US, the diagnosis of Barrett's esophagus requires the presence of intestinal-type epithelium with goblet cells since clinically only this type of esophageal columnar metaplasia predisposes to esophageal adenocarcinoma (3). While the annual risk of developing esophageal adenocarcinoma in Barrett's esophagus patients is low, approximately 0.15–0.5% per year, the lifetime risk is at least 10-fold higher than the general population (4). Since Barrett's esophagus represents the initial step in the stepwise malignant progression from metaplasia to low-grade dysplasia, high-grade dysplasia, and esophageal adenocarcinoma, identifying the cells that give rise to Barrett's esophagus could lead to more effective prevention and treatment strategies for both Barrett's esophagus and esophageal adenocarcinoma.

When esophageal squamous mucosa is chronically damaged by extrinsic factors such as intestinal bile and gastric acid, the normal stratified squamous epithelium may be replaced with a specialized intestinal columnar-lined epithelium containing goblet cells, which is known as Barrett's esophagus (5). Barrett's esophagus originates at the gastroesophageal junction (GEJ), the boundary between distal esophageal and gastric cardiac epithelial cells. There is an ongoing debate regarding the cell of origin of Barrett's esophagus and how esophageal epithelial metaplasia occurs (6). Previously, it was postulated that terminally differentiated esophageal squamous cells might undergo direct conversion into specialized intestinal columnar cells, a process called transdifferentiation (7). More recently, it is believed that epithelial stem or progenitor cells undergo molecular reprogramming to give rise to specialized intestinal columnar cells, a process called transcommitment (8). Based on numerous studies, there are four putative sources for the Barrett's esophagus cell of origin, though none has been proven definitively in human patients. The first possible cell of origin for Barrett's esophagus is a squamous stem or progenitor cell native to the esophagus (9). The second are epithelial stem or progenitor cells found in the ducts or acini of esophageal submucosal glands deep to the squamous epithelium (10). Third, circulating bone marrow-derived stem or progenitor cells could migrate to regions of epithelial injury and subsequently differentiate into columnar cells to repair the injury (11). Fourth, columnar stem or progenitor cells from either the squamocolumnar junction (SCJ) or gastric cardia could move proximally into regions of damaged squamous epithelium and undergo intestinal differentiation (12, 13). Identifying the cell of origin of Barrett's esophagus has implications for understanding the molecular mechanisms underlying the metaplastic process and thus, it remains both a relevant clinical and scientific question. Here, we aim to summarize the current knowledge and recent findings of origins of epithelial metaplasia in the esophagus, focusing on native esophageal cells since a separate chapter will focus on putative columnar stem or progenitor cells found at the GEJ. Also, due to the clinical observation that Barrett's

esophagus can recur in the proximal esophagus following esophagectomy when the GEJ and its cells have been removed (14), we propose that native esophageal stem or progenitor cells contribute to Barrett's esophagus under some circumstances. Finally, because bone marrow-derived stem or progenitor cells have only been shown to home to the esophagus in the setting of bone marrow transplant following a physiologically stressful preconditioning regimen (11, 15), we will not consider them further under physiologic reflux conditions.

Esophageal Squamous Epithelial Cell Transdifferentiation or Transcommitment

One hypothesis regarding the cell of origin of Barrett's esophagus is that molecular reprogramming of squamous cells found in the esophagus give rise to intestinalized columnar epithelial cells. Depending on the differentiation state of the original esophageal squamous cell, two different sub-hypotheses have developed. First, a fully differentiated squamous esophageal epithelial cell could undergo a "transdifferentiation" process resulting in a simple columnar epithelium reminiscent of the small intestine. Alternatively, normal squamous stem or progenitor cells could undergo reprogramming through a "transcommitment" process into columnar cells rather than squamous cells. Evidence to support either squamous transdifferentiation or transcommitment come from studies involving human Barrett's esophagus patients, a surgical model of induced gastroesophageal reflux in rats, and normal mouse esophageal epithelial development.

In support of the transdifferentiation process, Shields and colleagues identified a unique transition zone cell at the SCJ in Barrett's esophagus patients by scanning electron microscopy (16). Transition zone cells appeared to have ultrastructural features of both squamous and columnar epithelial cells such as squamous intercellular ridges covered with columnar microvilli. By light microscopy, a multilayered epithelium (MLE) was observed at the SCJ with 4 to 8 layers of distinct stratified squamous-like cells found below superficial mucin-containing epithelial cells with microvilli. These cells were visually different from Barrett's epithelial cells and normal esophageal squamous epithelial cells. More importantly, MLE was not found in biopsies taken from the normal physiologic GEJ, suggesting that these cells were unique to Barrett's esophagus in the gastrointestinal tract. A later study from the same group demonstrated that basal cells in MLE expressed both squamous cytokeratins (CK) 4 and 13 and columnar CK19 differentiation markers in line with the transdifferentiation premise (17). In a study from Chen and colleagues, MLE was observed at the neo-squamocolumnar junction as well as in the mid-esophagus in a rat model of Barrett's esophagus caused by surgically-induced gastroesophageal reflux via an esophagogastrroduodenal anastomosis (18). Since rats do not have submucosal glands in their esophagus, MLE in the mid-esophagus would most likely arise from a native esophageal squamous epithelial cell. MLE has, therefore, been suggested as an early or intermediate stage of columnar metaplasia.

Evidence for transcommitment of esophageal epithelial stem or progenitor cells comes from observations made during normal mouse esophageal development. Similar to human beings, the embryonic mouse esophagus is lined with a simple columnar epithelium which changes

during development to a stratified squamous epithelium. Columnar CK8 and CK18 differentiation markers are expressed in the earliest esophageal epithelial cells. As development proceeds, CK8 and CK18 expression decreases while basal squamous epithelial cells begin to express squamous marker CK14. Yu and colleagues from the Tosh lab developed an explant culture system to study the mechanism of this phenotypic switch (19). They utilized esophagi from day 11.5 mouse embryos and grew them in this culture system to mimic *in vivo* esophageal epithelial development. Using immunostaining for the columnar marker CK8 and squamous marker CK14 and a reporter gene using the CK14 promoter to drive green fluorescent protein (GFP) expression, they found that individual CK8 expressing esophageal epithelial cells could simultaneously express GFP or CK14 during later stages of esophageal development. This occurred despite treatment with apoptosis or cell division inhibitors and concluded with promoter methylation and epigenetic silencing of CK8. Together, these results showed that an individual esophageal epithelial cell could undergo a phenotypic switch from columnar to squamous epithelium during normal embryogenesis.

Esophageal Squamous Epithelial Stem or Progenitor Cells

Using various techniques, several studies have characterized mouse esophageal stem or progenitor cells by identifying cell surface makers and properties that differentiate various cell populations. Putative markers of esophageal stem or progenitor cells include the ability to exclude Hoechst dye (20); high expression of CD34 (21); high expression of Sca-1 and Thy-1 (22); the ability to retain 5-bromo-2'-deoxyuridine (BrdU) or tritiated thymidine (^3H -TdR) (21); high expression of $\alpha 6$ integrin, $\beta 4$ integrin, and CD73 (23); and low expression of CD71 (24). These markers identify cells that have clonogenic potential, the ability to form organoids *in vitro*, and the ability to regenerate a fully differentiated esophageal epithelium post injury. Doupe and colleagues in the Jones lab identified a population of label retaining cells (LRC), representing 0.4% of esophageal basal epithelium in mice, as esophageal progenitor cells (25). However, these LRC did not stain for CK14 or CD34 as previously reported for basal epithelium. Instead, these cells were CD45 positive consistent with a hematopoietic cell origin. More recently, Giroux and colleagues from the Rustgi lab identified a long-lived progenitor population in the basal epithelium of the mouse esophagus that expresses CK15 (26). CK15 pairs with CK5 in a subset of basal squamous esophageal cells, while CK14 pairs with CK5 in the vast majority of basal squamous esophageal cells. CK15 positive cells were able to give rise to adjacent Ki-67 positive proliferative basal cells as well as CK13 positive suprabasal cells. Expression analysis revealed that CK15 positive cells downregulated CD34 and upregulated $\alpha 6$ integrin and CD73. When grown in three-dimensional (3D) organoid culture, CK15 positive cells gave rise to more colonies and organoids that exhibited full normal differentiation including keratinization. Ablation of CK15 positive cells led to esophageal epithelial thinning as well as decreased numbers of Ki-67 positive cells. Finally, CK15 positive basal cells were shown to be radioresistant and to be responsible for mediating radiation-induced injury. These findings suggest that in mice CK15 marks a reserve stem cell population in the esophagus.

Human esophageal epithelium is non-keratinized and interrupted by folds of stromal papillae, which allows division of the epithelium into regions overlying stromal papillae or

regions between papillae. Multiple groups using different techniques and cell surface markers have reported inconsistent results in terms of stem and/or progenitor cell characteristics of papillary and interpapillary epithelium. For example, a recent study from the Fitzgerald lab investigated distinct populations of esophageal epithelium based on the expression of epithelial and progenitor cell markers (EpCAM and CD34) (27). Surprisingly, they found that cells at diverse stages of differentiation sorted according to progenitor cell markers have equal capacity for self-renewal and ability to reconstitute a squamous 3D architecture *in vitro*. Their data suggest that progenitor cells are more widespread throughout the human esophageal epithelium than previously thought. In contrast, an older study by Seery and Watt found that basal cells in the interpapillary regions that express integrin $\beta 1$ had increased clonogenic potential and divided asymmetrically as compared to basal cells overlying papillae (28). Human organotypic “esophagospheres” were established by Jeong and colleagues from the Diehn lab (29). Esophageal epithelium from endoscopic biopsies was dissociated using dispase and trypsin and grown in Matrigel and defined media. 3D structures formed in the absence of stromal components. When grown in “progenitor” media, clusters of uniform cells expressing CK14, integrin $\alpha 6$ (CD49f), and p63 appeared. If these clusters or original primary epithelial cells were grown in “sphere” media, circumferential stratified squamous epithelium formed an esophagosphere with keratinized cells in the center. While the outer cells expressed CK14, CD49f, and p63, inner cells appeared differentiated with expression of suprabasal CK4. These esophagospheres could be grown up to 300-fold in size and serially passaged. Further analysis demonstrated that CD49f high and CD24 low cells had the highest sphere-forming ability. Knockdown of P63 in these cells led to decreased sphere-forming ability.

Recently, evidence supporting basal esophageal epithelial cells as the origin of Barrett’s esophagus in mice and human beings has been reported. Jiang and colleagues from the Que lab induced hyperplastic glands at the mouse SCJ that resemble human MLE (30). This occurred when the transcription factor SOX2 was overexpressed in cells using the promoter of basal squamous CK5. Using elegant genetic lineage tracing techniques, they showed that this MLE originated from basal epithelial progenitor cells found at the mouse SCJ proximal to the gastric cardia that express the transcription factor P63, squamous CK5, and columnar CK7 (triple positive cells). These cells were found just distal to squamous basal cells that expressed P63 and CK5 but did not express CK7. When a surgical esophageal-duodenal anastomosis was created in mice with a P63 reporter gene, MLE was again observed suggesting that acidic bile reflux can induce the triple positive cells to undergo metaplasia. When the transcription factor CDX2 was overexpressed in cells expressing squamous CK5 or in cells expressing columnar CK7, the metaplasia now intestinalized with expression of intestinal markers Villin, TFF3, and MUC2. Finally, triple positive cells were shown to also exist at the human SCJ and this cell population is expanded in human patients with GERD and Barrett’s esophagus.

Esophageal Submucosal Glands or Ducts Transdifferentiation or Transcommitment

Submucosal glands are found in the human esophagus deep to the stratified squamous epithelium with ducts that penetrate the squamous epithelium and empty into the esophageal lumen. The glands secrete mucus, bicarbonate, and growth factors, and when injured can undergo proliferative acinar ductal metaplasia characterized by the expression of CK7 (31). Based on previous human and animal experimental data, esophageal submucosal glands and ducts could serve as a source of epithelial stem or progenitor cells that respond to esophageal injury (32). It is important to note that common laboratory animals such as mice and rats do not have esophageal submucosal glands, and as a result the development of Barrett's esophagus in these rodent models suggests a different cell of origin. To assess the contribution of submucosal glands and their ducts to Barrett's esophagus, less common animal models such as dogs and pigs have been used.

Using serial sections of human Barrett's esophagus, Coad and colleagues from the United Kingdom first studied the histologic relationship between esophageal gland ducts, Barrett's esophagus, and associated squamous islands (33). They found that squamous islands are generally connected to esophageal gland duct epithelium as are regions of columnar cells in patients with Barrett's esophagus. In addition, they noted that there was a gradual morphological change between the cells of the esophageal gland ducts and the Barrett's epithelium. In a follow-up report that correlated patient histology with molecular markers, Leedham and colleagues found that esophageal submucosal gland ducts not only have physical continuity with areas of columnar esophagus but they also share mutations with Barrett's esophagus epithelium (34). For example, a specific P16 point mutation identified in the epithelium of the esophageal gland duct was also present in a contiguous metaplastic crypt of Barrett's dysplasia, whereas neo-squamous islands arising from squamous ducts were P16 wild-type. This data from patients suggests that Barrett's esophagus can arise from esophageal submucosal gland duct epithelium.

Classic experiments in dogs show this more directly. Gillen and colleagues created a dog model of acid reflux-mediated epithelial damage following surgical cardioplasty and fixed hiatal hernias (10). Circumferential stripping of the squamous epithelium of the distal esophagus was then performed in two different 2 cm wide sections separated by a 2 cm section of intact squamous epithelium. To enhance acid secretion, pentagastrin was administered to some of the animals; while biliary surgery was performed in other animals to induce reflux of both acid and bile. The SCJ and distal esophagus were evaluated in all animals at the end of three months. Animals with a hiatal hernia which received pentagastrin or those with a hiatal hernia and biliary diversion had columnar epithelium containing goblet cells present in the lower denuded ring while control animals only had squamous epithelium present. Interestingly, one third of the animals with a hiatal hernia treated with pentagastrin developed columnar epithelium in the upper denuded ring, above the ring of intact squamous epithelium. These experiments led to the conclusions that SCJ columnar cells had not migrated proximally across an intact squamous epithelium and that the source of metaplastic

columnar cells was most likely submucosal glands and ducts since ducts contiguous with esophageal surface ulcerations were observed histologically.

Von Furstenberg and colleagues from the Garman lab recently reported their work examining porcine esophageal submucosal glands (35). Pigs are an ideal model to study esophageal submucosal glands as the density is much higher than in human beings. In an *in vivo* esophageal epithelial injury model using radiofrequency ablation (RFA), uptake of 5-ethynyl-2'-deoxyuridine (EdU) occurred in basal squamous epithelium when examined 7 days after RFA compared to control animals. While submucosal gland ducts did not differ significantly from control animals, EdU uptake was significantly increased in submucosal glands. Further, the glands appeared to undergo acinar ductal metaplasia with upregulation of CK7 expression. These investigators next isolated submucosal glands and placed them into 3D culture. Budding structures were observed after 7 days and these were subsequently digested into single cell clones. Single cell clones, which required epidermal growth factor (EGF), formed two types of spheroids within 5 days. Hollow spheroids expressed CK7 while solid spheroids expressed P63. Microarray analysis revealed that both spheroids were distinct from intact submucosal glands with increased cell proliferation, cell cycle, wound healing, and glandular gene expression. The authors concluded that submucosal gland isolation was similar to RFA in inducing injury repair pathways. When the spheroids were compared to normal esophageal squamous epithelium, increased expression of Barrett's esophagus associated genes such as SOX9, CK8, CK18, AGR2, MUC1, and MUC13 were observed. However, when compared to normal submucosal glands, only the mucins were more highly expressed in the spheroids.

Together, these studies strongly support the notion that stem or progenitor cells within esophageal submucosal glands and/or ducts can generate both squamous and Barrett's columnar cells. However, which exact cell type within the esophageal submucosal glands and their ducts serves as the source of Barrett's esophagus remains to be determined. Also, transcommitment must occur within these cells to give rise to the variety of differentiated intestinal lineages found in Barrett's esophagus.

Molecular Mediators of Transcommitment

Gene expression analysis of clonogenic cells derived from endoscopic biopsies of the distal esophagus, Barrett's esophagus, and proximal stomach from human patients shows that each are distinctly different from each other (36). Whatever the source of the Barrett's esophagus cell of origin, the current hypotheses do not explain how any of the putative epithelial stem or progenitor cells give rise to the various intestinal cell lineages found in human Barrett's esophagus, outside of molecular reprogramming. Mechanistically, an epithelial stem or progenitor cell that gives rise to Barrett's esophagus, regardless of its origin, would need to acquire or maintain a columnar phenotype, undergo intestinalization, and secrete mucus as goblet cells are the *sine qua non* of specialized intestinal metaplasia. Switching from a squamous to a columnar phenotype or maintaining a columnar phenotype could require the downregulation of transcription factors that characterize squamous cells (i.e., SOX2 and P63) and activation of transcription factors that characterize columnar cells (i.e., SOX9). This could be followed by activation of intestinal (i.e., CDX1 and CDX2) and mucin

associated transcription factors (i.e., FOXA2). In the tubular esophagus, two squamous transcription factors have been widely studied, P63 and SOX2. P63 is a member of the P53 family of transcription factors which has six isoforms. In addition to three full length proteins named TAp63, there are three other proteins transcribed from an alternate promoter in the third intron known as Np63, which do not contain the amino terminal transactivation domain but retain the carboxyl terminal DNA binding domain. Np63 is the predominant isoform expressed in esophageal squamous epithelium. Wang and colleagues from the McKeon lab have shown that embryonic P63 knockout mice develop a Barrett's like metaplasia at their SCJ (13). SOX2 hypomorphic mice have a variable esophageal epithelial phenotype with regions that appear columnar and mucin-producing (37). In contrast, the columnar transcription factor SOX9 was first identified in the GI tract within proliferative cells of intestinal crypts (38). Using esophageal epithelium isolated from wild type C57BL/6 mice in a 3D organotypic culture system, Clemons and colleagues from the Phillips lab found that retroviral transduction of SOX9 induced expression of columnar CK8 and of the intestinal protein A33 and changed the stratified squamous epithelium into one to two layers of cuboidal or columnar shaped epithelial cells (39). To determine the role of SOX9 in the development of Barrett's esophagus, we previously performed SOX9 immunohistochemical staining on esophageal tissue microarrays representing esophagectomy specimens from 96 individual patients containing Barrett's esophagus with high-grade dysplasia and/or esophageal adenocarcinoma (40). We found SOX9 expression in 100% of patients with Barrett's esophagus and in 85% of patients with esophageal adenocarcinoma but no SOX9 expression in adjacent squamous epithelium. Further studies revealed that SOX9 was activated in Barrett's epithelium through acid and bile-induced Hedgehog ligand secretion by epithelial cells that in turn activate BMP4 secretion by adjacent stromal cells. Stromal BMP4 then acts back on the epithelium to induce SOX9 expression.

Following acid and bile injury mediated molecular reprogramming, a metaplastic columnar cell could then upregulate intestinal transcription factors CDX1 and CDX2 to become an intestinal cell followed by upregulation of the mucin associated transcription factor FOXA2 to become a mucin secreting goblet cell. CDX1 and CDX2 are members of the caudal related homeobox gene family which are expressed in the intestine. Within the intestine CDX2 is a major transcriptional activator and thus, CDX2 loss and resultant loss of its downstream target genes seem to cause a reprogramming of intestinal progenitor cells into squamous cells. This was shown by Chawengsaksophak and colleagues when they examined CDX2 heterozygote mice (41). These mice developed intestinal polyps which contained regions of squamous epithelium in which the remaining CDX2 allele was shown to have been silenced. Furthermore, ultrastructural analysis of mouse esophageal basal epithelium in which CDX2 is ectopically expressed using the murine CK14 gene promoter showed that cells acquired some characteristics of MLE (42). We previously demonstrated that FOXA2 is expressed in Barrett's esophagus but not in normal esophageal squamous epithelium (43). Overexpression of FOXA2 in normal esophageal squamous epithelial cells induces expression of the intestinal mucin MUC2 as well as AGR2, a disulphide isomerase required for MUC2 processing and another marker of Barrett's epithelium.

These transcription factors can be regulated by upstream signaling pathways such as Hedgehog, BMP4, Notch, and Wnt. Our lab has previously shown that SOX9 and FOXA2

can be regulated by Hedgehog and downstream BMP4 signaling (40, 43). SOX9 is also a Wnt pathway target in intestinal crypts. Notch pathway components such as ATOH1 and DLL1 regulate both CDX2 and MUC2 expression in Barrett's esophagus(44, 45). In the rat esophagojejunostomy model to induce bile reflux, treatment of animals with a gamma-secretase inhibitor to block Notch signaling led to almost complete conversion of intestinal metaplasia into mucus-secreting goblet cells (46).

It is likely that other transcription factors that cannot be as easily classified as squamous, columnar, intestinal, or mucin-related may play a role in metaplasia as well. For example, investigators from the Rustgi lab used a combination of MYC and CDX1 overexpression with Notch pathway inhibition in the telomerase-immortalized human esophageal squamous epithelial cell line EPC2 (47). This led to downregulation of squamous cytokeratins and upregulation of columnar cytokeratins and mucins. More importantly, in 3D culture, elongated cells were observed in the basal layer of the epithelium with Notch inhibition as shown by light and electron microscopy. Finally, investigators from the Tosh lab recently demonstrated that overexpression of HNF4 α in esophageal epithelial cells led to expression of columnar CK8 and intestinal Villin and TFF3 (48).

Conclusions

The incidence of esophageal adenocarcinoma in Western countries has greatly increased in recent decades, with the only recognized precursor lesion being Barrett's esophagus. Identifying the Barrett's esophagus cell of origin would aid efforts to develop therapeutic interventions in patients with Barrett's esophagus to either halt or reverse metaplasia or malignant progression. While enthusiasm is building to support several different cell populations at the SCJ as the Barrett's esophagus cell of origin due to intriguing experimental results in mouse models, whether any of these cell populations represents the unique cell of origin of Barrett's esophagus in human patients remains unclear. This is because these mouse models do not explain several clinical observations made in patients. "Lineage tracing" in human patients using a P16 point mutation suggests that Barrett's esophagus can arise from esophageal submucosal glands while recurrence of Barrett's esophagus in the cervical esophagus following esophagectomy and partial gastrectomy suggest that Barrett's esophagus can arise from an esophageal squamous epithelial stem or progenitor cell. Based on this, we conclude that a native esophageal stem or progenitor cell is likely the cell of origin for Barrett's esophagus in at least some patients. At present, insights from novel mouse models and clinical observations in patients should be integrated in the search for the Barrett's esophagus cell of origin and to study the molecular mechanisms underlying esophageal columnar metaplasia. We cannot exclude that in individual patients the Barrett's esophagus cell of origin may differ based on clinical and physiologic differences. Thus, this clinical and scientific challenge still remains.

Acknowledgments

Funding: This work was supported by the U.S. National Institutes of Health, R01 DK097340 to DHW.

Abbreviations

GERD	gastroesophageal reflux disease
GEJ	gastroesophageal junction
SCJ	squamocolumnar junction
MLE	multilayered epithelium
CK	cytokeratin
GFP	green fluorescent protein
CD	cluster of differentiation
BRDU	5-bromo-2'-deoxyuridine
LRC	label retaining cell
3D	three dimensional
RFA	radiofrequency ablation
EdU	5-ethynyl-2'-deoxyuridine
EGF	epidermal growth factor

References

1. Rustgi AK, and El-Serag HB. Esophageal carcinoma. *N Engl J Med*. 2014;371(26):2499–509. [PubMed: 25539106]
2. Spechler SJ, and Souza RF. Barrett's esophagus. *N Engl J Med*. 2014;371(9):836–45. [PubMed: 25162890]
3. Spechler SJ, Sharma P, Souza RF, Inadomi JM, and Shaheen NJ. American Gastroenterological Association technical review on the management of Barrett's esophagus. *Gastroenterology*. 2011;140(3):e18–52; quiz e13. [PubMed: 21376939]
4. Solaymani-Dodaran M, Logan RF, West J, Card T, and Coupland C. Risk of oesophageal cancer in Barrett's oesophagus and gastro-oesophageal reflux. *Gut*. 2004;53(8):1070–4. [PubMed: 15247170]
5. Spechler SJ, Fitzgerald RC, Prasad GA, and Wang KK. History, molecular mechanisms, and endoscopic treatment of Barrett's esophagus. *Gastroenterology*. 2010;138(3):854–69. [PubMed: 20080098]
6. Xian W, Ho KY, Crum CP, and McKeon F. Cellular origin of Barrett's esophagus: controversy and therapeutic implications. *Gastroenterology*. 2012;142(7):1424–30. [PubMed: 22537611]
7. Eberhard D, and Tosh D. Transdifferentiation and metaplasia as a paradigm for understanding development and disease. *Cell Mol Life Sci*. 2008;65(1):33–40. [PubMed: 18030422]
8. Wang DH, and Souza RF. Transcommitment: Paving the Way to Barrett's Metaplasia. *Adv Exp Med Biol*. 2016;908(183–212. [PubMed: 27573773]
9. Stairs DB, Nakagawa H, Klein-Szanto A, Mitchell SD, Silberg DG, Tobias JW, Lynch JP, and 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(10):e3534. [PubMed: 18953412]
10. Gillen P, Keeling P, Byrne PJ, West AB, and Hennessy TP. Experimental columnar metaplasia in the canine oesophagus. *Br J Surg*. 1988;75(2):113–5. [PubMed: 3349294]

11. Sarosi G, Brown G, Jaiswal K, Feagins LA, Lee E, Crook TW, Souza RF, Zou YS, Shay JW, and Spechler SJ. Bone marrow progenitor cells contribute to esophageal regeneration and metaplasia in a rat model of Barrett's esophagus. *Dis Esophagus*. 2008;21(1):43–50. [PubMed: 18197938]
12. Quante M, Bhagat G, Abrams JA, Marache F, Good P, Lee MD, Lee Y, Friedman R, Asfaha S, Dubeykovskaya Z, et al. Bile Acid and inflammation activate gastric cardia stem cells in a mouse model of barrett-like metaplasia. *Cancer Cell*. 2012;21(1):36–51. [PubMed: 22264787]
13. Wang X, Ouyang H, Yamamoto Y, Kumar PA, Wei TS, Dagher R, Vincent M, Lu X, Bellizzi AM, Ho KY, et al. Residual embryonic cells as precursors of a Barrett's-like metaplasia. *Cell*. 2011;145(7):1023–35. [PubMed: 21703447]
14. Oberg S, Johansson J, Wenner J, and Walther B. Metaplastic columnar mucosa in the cervical esophagus after esophagectomy. *Ann Surg*. 2002;235(3):338–45. [PubMed: 11882755]
15. Hutchinson L, Stenstrom B, Chen D, Piperdi B, Levey S, Lyle S, Wang TC, and Houghton J. Human Barrett's adenocarcinoma of the esophagus, associated myofibroblasts, and endothelium can arise from bone marrow-derived cells after allogeneic stem cell transplant. *Stem cells and development*. 2011;20(1):11–7. [PubMed: 20677919]
16. Shields HM, Zwas F, Antonioli DA, Doos WG, Kim S, and Spechler SJ. Detection by scanning electron microscopy of a distinctive esophageal surface cell at the junction of squamous and Barrett's epithelium. *Dig Dis Sci*. 1993;38(1):97–108. [PubMed: 8420766]
17. Boch JA, Shields HM, Antonioli DA, Zwas F, Sawhney RA, and Trier JS. Distribution of cytokeratin markers in Barrett's specialized columnar epithelium. *Gastroenterology*. 1997;112(3):760–5. [PubMed: 9041237]
18. Chen X, Qin R, Liu B, Ma Y, Su Y, Yang CS, Glickman JN, Odze RD, and Shaheen NJ. Multilayered epithelium in a rat model and human Barrett's esophagus: similar expression patterns of transcription factors and differentiation markers. *BMC Gastroenterol*. 2008;8(1). [PubMed: 18190713]
19. Yu WY, Slack JM, and Tosh D. Conversion of columnar to stratified squamous epithelium in the developing mouse oesophagus. *Dev Biol*. 2005;284(1):157–70. [PubMed: 15992795]
20. Epperly MW, Guo H, Shen H, Niu Y, Zhang X, Jefferson M, Sikora CA, and Greenberger JS. Bone marrow origin of cells with capacity for homing and differentiation to esophageal squamous epithelium. *Radiat Res*. 2004;162(3):233–40. [PubMed: 15333000]
21. Kalabis J, Oyama K, Okawa T, Nakagawa H, Michaylira CZ, Stairs DB, Figueiredo JL, Mahmood U, Diehl JA, Herlyn M, et al. A subpopulation of mouse esophageal basal cells has properties of stem cells with the capacity for self-renewal and lineage specification. *The Journal of clinical investigation*. 2008;118(12):3860–9. [PubMed: 19033657]
22. Epperly MW, Shen H, Jefferson M, and Greenberger JS. In vitro differentiation capacity of esophageal progenitor cells with capacity for homing and repopulation of the ionizing irradiation-damaged esophagus. *In Vivo*. 2004;18(6):675–85. [PubMed: 15646807]
23. DeWard AD, Cramer J, and Lagasse E. Cellular heterogeneity in the mouse esophagus implicates the presence of a nonquiescent epithelial stem cell population. *Cell reports*. 2014;9(2):701–11. [PubMed: 25373907]
24. Croagh D, Phillips WA, Redvers R, Thomas RJ, and Kaur P. Identification of candidate murine esophageal stem cells using a combination of cell kinetic studies and cell surface markers. *Stem Cells*. 2007;25(2):313–8. [PubMed: 17038667]
25. Doupe DP, Alcolea MP, Roshan A, Zhang G, Klein AM, Simons BD, and Jones PH. A single progenitor population switches behavior to maintain and repair esophageal epithelium. *Science*. 2012;337(6098):1091–3. [PubMed: 22821983]
26. Giroux V, Lento AA, Islam M, Pitarresi JR, Kharbanda A, Hamilton KE, Whelan KA, Long A, Rhoades B, Tang Q, et al. Long-lived keratin 15+ esophageal progenitor cells contribute to homeostasis and regeneration. *The Journal of clinical investigation*. 2017;127(6):2378–91. [PubMed: 28481227]
27. Barbera M, di Pietro M, Walker E, Brierley C, MacRae S, Simons BD, Jones PH, Stingl J, and Fitzgerald RC. The human squamous oesophagus has widespread capacity for clonal expansion from cells at diverse stages of differentiation. *Gut*. 2015;64(1):11–9. [PubMed: 24572143]

28. Seery JP, and Watt FM. Asymmetric stem-cell divisions define the architecture of human oesophageal epithelium. *Current biology : CB*. 2000;10(22):1447–50. [PubMed: 11102807]
29. Jeong Y, Rhee H, Martin S, Klass D, Lin Y, Nguyen le XT, Feng W, and Diehn M. Identification and genetic manipulation of human and mouse oesophageal stem cells. *Gut*. 2016;65(7):1077–86. [PubMed: 25897018]
30. Jiang M, Li H, Zhang Y, Yang Y, Lu R, Liu K, Lin S, Lan X, Wang H, Wu H, et al. Transitional basal cells at the squamous-columnar junction generate Barrett's oesophagus. *Nature*. 2017;550(7677):529–33. [PubMed: 29019984]
31. Garman KS, Kruger L, Thomas S, Swiderska-Syn M, Moser BK, Diehl AM, and McCall SJ. Ductal metaplasia in oesophageal submucosal glands is associated with inflammation and oesophageal adenocarcinoma. *Histopathology*. 2015;67(6):771–82. [PubMed: 25847432]
32. Garman KS. Origin of Barrett's Epithelium: Esophageal Submucosal Glands. *Cell Mol Gastroenterol Hepatol*. 2017;4(1):153–6. [PubMed: 28593186]
33. Coad RA, Woodman AC, Warner PJ, Barr H, Wright NA, and Shepherd NA. On the histogenesis of Barrett's oesophagus and its associated squamous islands: a three-dimensional study of their morphological relationship with native oesophageal gland ducts. *The Journal of pathology*. 2005;206(4):388–94. [PubMed: 15926200]
34. Leedham SJ, Preston SL, McDonald SA, Elia G, Bhandari P, Poller D, Harrison R, Novelli MR, Jankowski JA, and Wright NA. Individual crypt genetic heterogeneity and the origin of metaplastic glandular epithelium in human Barrett's oesophagus. *Gut*. 2008;57(8):1041–8. [PubMed: 18305067]
35. von Furstenberg RJ, Li J, Stolarchuk C, Feder R, Campbell A, Kruger L, Gonzalez LM, Blikslager AT, Cardona DM, McCall SJ, et al. Porcine Esophageal Submucosal Gland Culture Model Shows Capacity for Proliferation and Differentiation. *Cell Mol Gastroenterol Hepatol*. 2017;4(3):385–404. [PubMed: 28936470]
36. Yamamoto Y, Wang X, Bertrand D, Kern F, Zhang T, Duleba M, Srivastava S, Khor CC, Hu Y, Wilson LH, et al. Mutational spectrum of Barrett's stem cells suggests paths to initiation of a precancerous lesion. *Nature communications*. 2016;7(10380).
37. Que J, Okubo T, Goldenring JR, Nam KT, Kurotani R, Morrissey EE, Taranova O, Pevny LH, and Hogan BL. Multiple dose-dependent roles for Sox2 in the patterning and differentiation of anterior foregut endoderm. *Development*. 2007;134(13):2521–31. [PubMed: 17522155]
38. Blache P, van de Wetering M, Duluc I, Domon C, Berta P, Freund JN, Clevers H, and Jay P. SOX9 is an intestine crypt transcription factor, is regulated by the Wnt pathway, and represses the CDX2 and MUC2 genes. *J Cell Biol*. 2004;166(1):37–47. [PubMed: 15240568]
39. Clemons NJ, Wang DH, Croagh D, Tikoo A, Fennell CM, Murone C, Scott AM, Watkins DN, and Phillips WA. Sox9 drives columnar differentiation of esophageal squamous epithelium: a possible role in the pathogenesis of Barrett's esophagus. *Am J Physiol Gastrointest Liver Physiol*. 2012;303(12):G1335–46. [PubMed: 23064761]
40. Wang DH, Clemons NJ, Miyashita T, Dupuy AJ, Zhang W, Szczepny A, Corcoran-Schwartz IM, Wilburn DL, Montgomery EA, Wang JS, et al. Aberrant epithelial-mesenchymal Hedgehog signaling characterizes Barrett's metaplasia. *Gastroenterology*. 2010;138(5):1810–22. [PubMed: 20138038]
41. Chawengsaksophak K, James R, Hammond VE, Kontgen F, and Beck F. Homeosis and intestinal tumours in Cdx2 mutant mice. *Nature*. 1997;386(6620):84–7. [PubMed: 9052785]
42. Kong J, Crissey MA, Funakoshi S, Kreindler JL, and Lynch JP. Ectopic Cdx2 expression in murine esophagus models an intermediate stage in the emergence of Barrett's esophagus. *PLoS One*. 2011;6(4):e18280. [PubMed: 21494671]
43. Wang DH, Tiwari A, Kim ME, Clemons NJ, Regmi NL, Hodges WA, Berman DM, Montgomery EA, Watkins DN, Zhang X, et al. Hedgehog signaling regulates FOXA2 in esophageal embryogenesis and Barrett's metaplasia. *The Journal of clinical investigation*. 2014;124(9):3767–80. [PubMed: 25083987]
44. Tamagawa Y, Ishimura N, Uno G, Yuki T, Kazumori H, Ishihara S, Amano Y, and Kinoshita Y. Notch signaling pathway and Cdx2 expression in the development of Barrett's esophagus. *Lab Invest*. 2012;92(6):896–909. [PubMed: 22449796]

45. Tamagawa Y, Ishimura N, Uno G, Aimi M, Oshima N, Yuki T, Sato S, Ishihara S, and Kinoshita Y. Bile acids induce Delta-like 1 expression via Cdx2-dependent pathway in the development of Barrett's esophagus. *Lab Invest.* 2016;96(3):325–37. [PubMed: 26568294]
46. Menke V, van Es JH, de Lau W, van den Born M, Kuipers EJ, Siersema PD, de Bruin RW, Kusters JG, and Clevers H. Conversion of metaplastic Barrett's epithelium into post-mitotic goblet cells by gamma-secretase inhibition. *Dis Model Mech.* 2010;3(1–2):104–10. [PubMed: 20075383]
47. Vega ME, Giroux V, Natsuizaka M, Liu M, Klein-Szanto AJ, Stairs DB, Nakagawa H, Wang KK, Wang TC, Lynch JP, et al. Inhibition of Notch signaling enhances transdifferentiation of the esophageal squamous epithelium towards a Barrett's-like metaplasia via KLF4. *Cell Cycle.* 2014;13(24):3857–66. [PubMed: 25558829]
48. Colleypriest BJ, Burke ZD, Griffiths LP, Chen Y, Yu WY, Jover R, Bock M, Biddlestone L, Quinlan JM, Ward SG, et al. Hnf4alpha is a key gene that can generate columnar metaplasia in oesophageal epithelium. *Differentiation.* 2017;93(39–49. [PubMed: 27875772]

Key Findings/Future Unmet Needs/Implications for Clinicians

1. Identifying the cell of origin of Barrett's esophagus has implications for understanding the molecular mechanisms underlying the metaplastic process and could lead to more effective prevention and treatment strategies for both Barrett's esophagus and esophageal adenocarcinoma.
2. The identity of the Barrett's esophagus cell of origin has not been definitively shown in human patients.
3. Based on clinical observations, a native esophageal stem or progenitor cell located in the squamous epithelium and/or the submucosal glands or their ducts likely is the cell of origin of Barrett's esophagus in at least some patients.
4. Whatever the identity of the cell of origin, molecular reprogramming of any of the current putative sources must occur to give rise to the various intestinal cell lineages seen in Barrett's esophagus.
5. Transcommitment is molecular reprogramming of a stem or progenitor cell while transdifferentiation is molecular reprogramming of a fully differentiated cell.