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The Esophageal Squamous Epithelial Cell—Still a Reasonable Candidate for the Barrett's Esophagus Cell of Origin?

Barrett's esophagus is the metaplastic change of the squamous epithelium lining the distal esophagus into an intestinalized columnar epithelium that predisposes to esophageal adenocarcinoma development. The cell that gives rise to Barrett's esophagus has not been identified definitively, although several sources for the Barrett's esophagus cell of origin have been postulated. One possible source is a fully differentiated squamous epithelial cell or a squamous epithelial progenitor or stem cell native to the esophagus that, through molecular reprogramming, either transdifferentiation or transcommitment, could give rise to an intestinalized columnar cell. Multilayered epithelium found in human patients and rodents with Barrett's esophagus and direct phenotypic conversion of mouse embryonic esophageal epithelium provide support for this. Limitations in current experimental approaches may explain why it has been difficult to fully change an esophageal squamous epithelial cell into an intestinalized columnar cell in vitro. *(Cell Mol Gastroenterol Hepatol 2017;4:157–160; http://dx.doi.org/10.1016/j.jcmgh.2017.01.015)*

Barrett's esophagus is the metaplasia in which a columnar epithelium with intestinal features and characterized by the presence of goblet cells replaces the normal stratified squamous epithelium lining the distal esophagus.[1] This condition is important clinically because it increases the risk for developing esophageal adenocarcinoma.[1] Barrett's esophagus is thought to occur secondarily to chronic epithelial injury and accompanying inflammation caused by gastroesophageal reflux. Despite intense research efforts, the molecular mechanisms underlying this metaplastic change in epithelial phenotype have not been elucidated completely. Furthermore, the identity of the cell that gives rise to Barrett's esophagus has not been identified definitively. Identifying this cell of origin is essential because it has implications both for pathogenesis and treatment, especially in the setting of recurrent Barrett's esophagus after endoscopic ablation therapy.[1]

There are several postulated sources for the cell of origin in Barrett's esophagus.[1] An early hypothesis of how Barrett's esophagus forms was that damaged squamous epithelium was simply replaced by proximally migrating columnar epithelial cells from either the squamocolumnar junction or gastric cardia. When gastroesophageal reflux was induced surgically in dogs and metaplastic columnar epithelium subsequently was found in an area denuded of epithelium above a residual squamous epithelial barrier, focus shifted to identifying a cell of origin native to the esophagus.[2] Given that the normal epithelium found in the human esophagus is predominantly squamous (the exception being the epithelium lining submucosal gland ducts and comprising the submucosal glands), 2 distinct hypotheses developed on how a squamous epithelial cell could give rise to a columnar epithelial cell. First, a fully differentiated squamous epithelial cell could undergo irreversible direct phenotypic conversion through molecular reprogramming into an intestinalized columnar cell without undergoing mitosis, a process termed *transdifferentiation*. Alternatively, a squamous epithelial precursor or stem cell could undergo molecular reprogramming leading to a change in the cell fate of progeny cells, a process termed *transcommitment*. The other potential source for the Barrett's esophagus cell of origin besides a proximally migrating columnar epithelial cell, a native squamous epithelial cell, or a native epithelial cell from an esophageal submucosal gland or duct, is an external circulating stem cell (ie, from the bone marrow).

Evidence for transdifferentiation or transcommitment of a squamous cell comes from studying tissue obtained from human patients with Barrett's esophagus and from rats that develop esophageal columnar metaplasia after the surgical induction of gastroesophageal reflux, and observations made during normal mouse esophageal development. In human patients, identification of a distinctive transition zone cell at the junction of squamous epithelium and Barrett's epithelium was reported by Shields et al.[3] By scanning electron microscopy, these cells had ultrastructural features of both squamous and columnar epithelial cells. For example, they showed intercellular ridges, a characteristic feature of squamous cells, and short microvilli and bulging mucus, a characteristic feature of secretory columnar cells.

Importantly, these cells clearly were different from Barrett's epithelial cells and normal gastroesophageal junction cells. By light microscopy, the junction of squamous and columnar epithelium in patients with gastroesophageal reflux disease often showed a multilayered epithelium with mucus-producing columnar cells overlying immature squamous cells. Further studies showed that basal cells in multilayered epithelium simultaneously expressed columnar cytokeratin 19 and squamous cytokeratin 4.[4] Interestingly, multilayered epithelium also was observed in rats that had undergone a surgical procedure to induce bile reflux. In those rats that developed Barrett's esophagus in this setting, a multilayered epithelium was observed both at the neosquamocolumnar junction as well as in the midesophagus.[5] The finding of multilayered epithelium at the junction of squamous and columnar epithelium is consistent with multilayered epithelium representing an intermediate stage between squamous and Barrett's epithelium. Furthermore, because rats do not possess submucosal glands, multilayered epithelium, especially in the midesophagus, would appear to arise from a native esophageal squamous epithelial cell.

Similar to human beings, the mouse embryonic esophagus initially is lined by columnar epithelium that undergoes stratification and squamous differentiation during embryonic development. Initially, esophageal epithelial cells express the columnar cytokeratins 8 and 18. As development progresses, the expression of cytokeratins 8 and 18 diminish while basal squamous epithelial cells begin to express the squamous cytokeratin 14. Investigators from the Tosh laboratory developed an explant culture system to study this process more closely.[6] Esophagi isolated from day 11.5 mouse embryos and grown in this culture system mimicked esophageal epithelial development observed in vivo. By using immunostaining for cytokeratin 8 and a cytokeratin 14–green fluorescent protein reporter, these investigators found that as esophageal development progressed, individual esophageal epithelial cells expressing cytokeratin 8 began simultaneously to express green fluorescent protein, or cytokeratin 14. This occurred even in the presence of inhibitors of apoptosis or cell division and ended with epigenetic silencing of cytokeratin 8 by promoter methylation. These results showed that an individual esophageal epithelial cell could undergo a direct phenotypic conversion from columnar to squamous. Reversing this process theoretically could lead to a squamous cell giving rise to a Barrett's esophagus–like phenotype.

The difference between transdifferentiation and transcommitment depends on the differentiation status of the cell of origin. Multiple studies have identified discrete cell populations from the mouse esophagus that appear to have progenitor cell properties such as the ability to form colonies, give rise to organoids, and repopulate a fully differentiated esophageal epithelium after injury. Various markers to identify these cells include the exclusion of Hoescht dye, Sca-1 positivity, Thy-1 positivity, the ability to retain bromodeoxyuridine or tritiated thymidine, and the expression of α6 integrin, β4 integrin, CD71, and/or CD73 (reviewed by Wang and Souza[1]). Investigators from the Jones laboratory recently found that although mouse esophageal epithelium contained squamous progenitor cells that were functionally equivalent, quiescent label-retaining stem cells were not present.[7] Although mouse esophageal epithelium is keratinized and uniformly 4–5 cell layers thick, human esophageal epithelium is nonkeratinized, is interrupted by slender folds of stromal papillae, and typically is much thicker than mouse esophageal epithelium (Figure 1). Because of the papillae, human esophageal epithelium can be divided into portions overlying stromal papillae or portions overlying interpapillary regions. Various groups using different techniques have reported conflicting characteristics of these regions in regards to the proliferative and stem cell compartments (reviewed by Wang and Souza[1]). Although all groups agreed that the basal cells are the most proliferative, they disagreed as to whether the basal cells overlying the papillae or those found in the interpapillary regions undergo asymmetric vs symmetric division, retain iododeoxyuridine or tritiated thymidine, or give rise to Ki-67–expressing proliferating cells. More recently, investigators from the Fitzgerald laboratory sorted human esophageal epithelium from esophagectomy specimens using antibodies against CD34 (to mark basal cells) and epithelial cell adhesion molecule (to mark suprabasal cells) into 4 separate fractions.[8] In colony-forming assays and 3-dimensional (3D) organotypic cultures, all 4 fractions of cells had similar characteristics, leading to the conclusion that proliferative cells were widespread throughout the human esophageal epithelium.

Although the identity of stem cells in the human esophageal squamous epithelium continues to be debated, most agree that the Barrett's esophagus cell of origin must undergo some type of phenotypic change to acquire the characteristics of intestinal differentiation. In vitro experiments using differentiated human esophageal squamous epithelial cells showed that they can undergo molecular reprogramming. Treatment with acidified media and/or bile salts, mimicking gastroesophageal reflux conditions, led to down-regulation of squamous transcription factors (eg, ΔNp63), and up-regulation of columnar (eg, SOX9) and intestinal (eg, CDX1, CDX2, and FOXA2) transcription factors, as well as alterations in various signaling pathways (reviewed by Wang and Souza[1]). These transcription factors are classified as such because they are expressed by squamous, columnar, and intestinal mucus-producing epithelial cells and have been shown to regulate markers of squamous, columnar, and intestinal mucus–producing differentiation. For example, the squamous transcription factor ΔNp63 up-regulated expression of the squamous cytokeratins 5 and 14, the columnar transcription factor SOX9 induced expression of columnar cytokeratins 8 and 18, and the intestinal transcription factors CDX1 and CDX2 and the mucus transcription factor FOXA2 induced expression of the intestinal mucin MUC2 in immortalized human esophageal squamous epithelial cells (reviewed by Wang and Souza[1]). Although many studies have depended on expression analyses to show a phenotypic change, novel 3D organotypic culture systems and electron microscopy have shown changes in cellular morphology after molecular

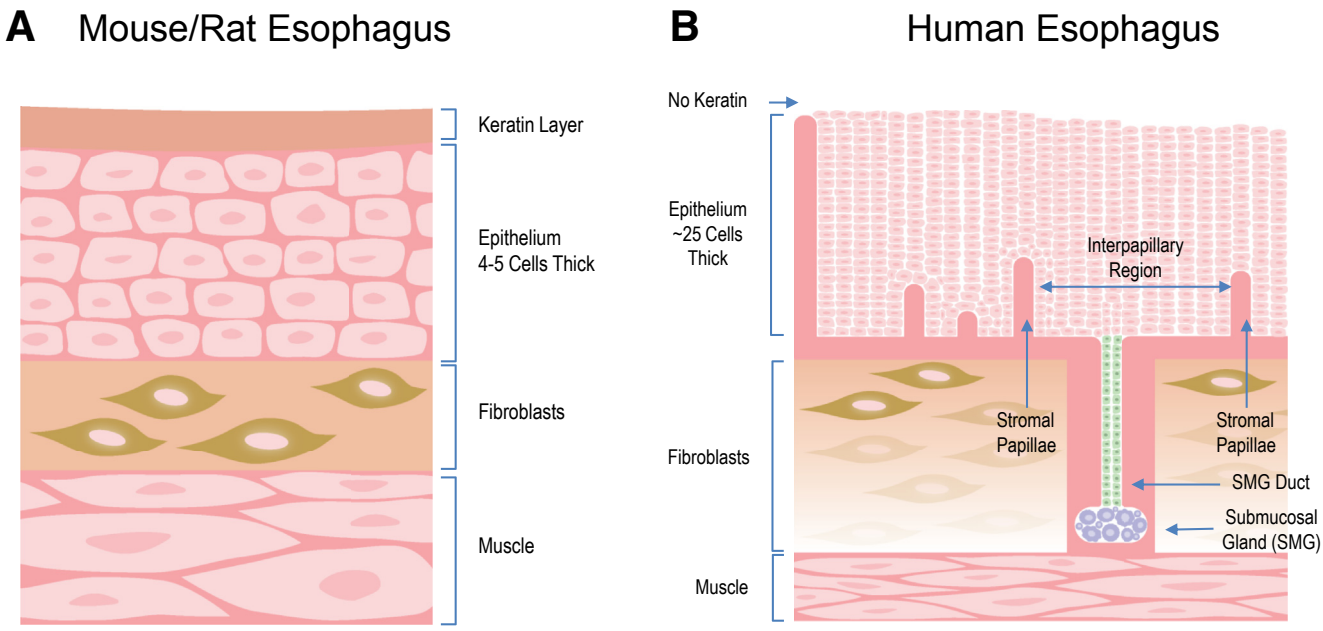

Figure 1. Schematic representation of the histologic structure of the mouse/rat and human esophagus. (*A*) The mouse/rat esophageal epithelium is keratinized stratified squamous and comprises 4–5 cell layers. Fibroblasts and muscle are located deep to the epithelium. Submucosal glands are absent. (*B*) The human esophageal epithelium is nonkeratinized stratified squamous and comprises many cell layers. Stromal papillae divide the epithelium into regions overlying papillae and interpapillary regions. Secretions made by submucosal glands are carried by ducts, lined by cuboidal cells (shown in green), and released into the esophageal lumen. Fibroblasts and muscle are located deep to the epithelium. Created by Medical Media, Dallas Veterans Affairs Medical Center.

reprogramming of human esophageal squamous epithelial cells, especially when multiple genetic alterations are induced simultaneously. For example, investigators from the Rustgi laboratory combined MYC and CDX1 overexpression with Notch pathway inhibition in the telomerase-immortalized human esophageal squamous epithelial cell line EPC2.[9] This led to down-regulated expression of squamous cytokeratins and up-regulated expression of columnar cytokeratins and mucins. More importantly, in 3D organotypic cultures, basal cells with these 3 genetic alterations appeared morphologically different and more elongated as shown by light and electron microscopy.

In summary, is the esophageal squamous epithelial cell still a reasonable candidate for the Barrett's esophagus cell of origin? Enthusiasm recently has shifted toward proximally migrating columnar cells from the squamocolumnar junction or gastric cardia based on intriguing data from genetic mouse models as well as toward submucosal glands and their ducts based on lineage tracing with P53 and P16 point mutations in human tissue specimens (reviewed by Wang and Souza[1]). However, the presence of epithelial cells that simultaneously express both squamous and columnar cytokeratins in vivo in both the human and rodent esophagus in the setting of gastroesophageal reflux suggests an initial squamous source for Barrett's esophagus, if multilayered epithelium truly represents an intermediate stage between squamous and Barrett's epithelium. In addition, the presence of multilayered epithelium in the midesophagus of rats, which do not have esophageal submucosal glands, after reflux-inducing surgery argues strongly against submucosal glands, their ducts, or a proximally migrating columnar cell as a source of the multilayered epithelium.[5]

If an esophageal squamous epithelial cell remains as a strong candidate for the Barrett's esophagus cell of origin, the next question is why an esophageal squamous epithelial cell has yet to be changed into an intestinalized goblet cell in vitro. This is a difficult question to answer but likely is owing to limitations in our current experimental approaches. First, we may not be using the correct esophageal squamous cell as a substrate for transdifferentiation or transcommitment experiments. Almost all studies in human cell lines have been performed in differentiated, immortalized cell lines, or in proliferative primary cell lines. Perhaps these cell lines do not contain the requisite squamous progenitor or stem cell with the plasticity to become an intestinalized columnar cell. Organoid cultures of esophageal squamous epithelium freshly isolated from patients may allow genetic manipulation of cells with the required plasticity. Second, phenotype switching from squamous to intestinalized columnar may require multiple genetic alterations in a specific combination and sequence. To date, the majority of studies have examined the effects of altering the expression of a single gene. A more logical approach perhaps is to stably express a columnar transcription factor, followed by an intestinal transcription factor, followed by a mucus-related transcription factor. Based on metaplasia in the pancreas where structural components have to be down-regulated as well as up-regulated, down-regulation of

squamous genes also may need to be incorporated into this sequence.[10] Third, proper culture conditions for cells to undergo transdifferentiation or transcommitment may be underutilized. Novel culture systems with an air–liquid interface and fibroblasts to permit epithelial–stromal interactions, such as 3D organotypic culture or in vivo transplant culture using a scaffold such as a denuded rat trachea, might be required to induce recognizable morphologic features of squamous or columnar differentiation, or even gland formation.

Finally, while renewing our focus on esophageal squamous epithelial cells as a potential source for the Barrett's esophagus cell of origin, we should not ignore novel insights gained from ongoing studies examining proximally migrating columnar cells in genetic mouse models as well as cells derived from esophageal submucosal glands and their ducts. Perhaps each of these cells eventually may be shown to be the source of the Barrett's esophagus cell of origin in different patients. None of them have been disproved or proved convincingly to date.

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