Cellular origin of Barrett’s metaplasia and oesophageal stem cells

Mariagnese Barbera and Rebecca C. Fitzgerald
Hutchison/MRC Research Centre, Hills Road, Cambridge CB2 0XZ, U.K.

Abstract
Barrett’s oesophagus is a metaplastic pre-malignant disorder and the only established precursor lesion for oesophageal adenocarcinoma. Barrett’s oesophagus develops when the normal stratified squamous epithelium of the lower oesophagus is replaced by a columnar lined mucosa with intestinal differentiation, usually in the context of chronic gastro-oesophageal reflux disease. The cellular and molecular mechanisms by which this metaplastic transformation occurs are poorly understood. Abnormal differentiation of multipotent stem cells in the squamous oesophagus, triggered by exposure to refluxate, is one potential mechanism. These stem cells could be located in the basal layer of the squamous oesophageal epithelium and/or in the neck region of the oesophageal submucosal gland ducts; however, their exact location and identification are still matter of discussion. Three-dimensional models combined with state-of-the-art imaging techniques are now applied to characterize the squamous epithelium in human oesophageal samples, and this could unveil essential information to identify these progenitor cells. Locating stem cells in human squamous oesophagus could have important implications for our understanding of Barrett’s oesophagus and remarkably improve our future strategies for its prevention.

Introduction
Metaplasia is the process whereby an adult tissue type transforms into another. Because this process is commonly associated with increased and disordered cell proliferation, metaplasia may often progress to dysplasia and, eventually, cancer. The Barrett’s oesophageal epithelium, which is a malignant precursor of OAC (oesophageal adenocarcinoma), is a typical example of this sequence. Barrett’s oesophagus occurs when the squamous multilayered lining of the lower oesophagus is replaced with a single layer of columnar mucosa in response to chronic gastro-oesophageal reflux. The differentiation may be along gastric or intestinal lineages and is therefore histopathologically subclassified into these two subtypes. Internationally, the definition of Barrett’s oesophagus tends to be restricted to the intestinal type, which has the clearest association with OAC. Overall, patients with Barrett’s oesophagus have a 30–50-fold increased risk of cancer compared with the general population [1,2].

In general, metaplasia can arise from wound healing, prolonged tissue stress or in response to abnormal tissue stimulation [3]. The importance of the luminal environment, and in particular reflux components, as a trigger for Barrett’s oesophagus has been widely established (e.g. [4]). However, the cellular and molecular mechanisms involved in Barrett’s oesophagus development are still poorly understood, and this is at least in part due to the difficulties in observing this process in vivo and the lack of reliable animal models. In particular, the cell origin of Barrett’s oesophagus is still controversial, and several hypotheses have been proposed to define and identify the cells which gives rise to the metaplastic tissue. Some of the original hypotheses have been almost dismissed in recent years, and none has, so far, been supported by definitive evidence.

The ‘gastric epithelium migration’ and ‘transdifferentiation’ theories
Initially, Barrett’s oesophagus was thought to arise as a consequence of upward cell migration from the transitional zone cells of the gastro-oesophageal junction [5] and it was proposed that these cells would migrate and colonize the distal oesophagus or the gastric cardia, in response to tissue damage from continued toxic exposure to refluxate (Figure 1a). A study on the distribution of oesophageal and gastric cardiac mucosae in oesophagectomy specimens led to the suggestion that cardiac glands would be exposed to the luminal surface and become columnar epithelial islands which could clonally expand to give rise to Barrett’s oesophagus [6]. However, in animal models, columnar epithelium can still develop in artificially defective mucosa above a squamous barrier, which separates the distal oesophagus from the transitional zone of the stomach [7]. Furthermore, even if the refluxate-mediated damage of the gastric cells can lead to their upward migration, the different epithelial cell lineages in Barrett’s oesophagus still need to be accounted for.

The second hypothesis assigns the origin of Barrett’s oesophagus to a subclass of metaplasia called ‘transdifferentiation’ (Figure 1b). This term describes an irreversible
metaplastic conversion from one fully differentiated state into another [3]. This hypothesis is supported by a study of the conversion of the murine epithelium from columnar into stratified squamous, during the development of the embryonic oesophagus [8]. It was shown that a proportion of cells co-express markers of squamous (cytokeratin 14) and columnar (cytokeratin 8) differentiation during the columnar–squamous conversion. The authors suggest that squamous oesophageal cells can arise directly from columnar basal cells, independent of cell division or apoptosis [8]. Thus the reverse transformation could account for the switch in phenotype in Barrett’s oesophagus. However, whether this switch can occur in adulthood remains to be proven. Furthermore, evidence of new squamous epithelium which develops after the endoscopic removal of Barrett’s oesophagus epithelium (e.g. [9]) (assuming that the differentiated epithelium was completely removed) would weaken the theory of transdifferentiation. A related hypothesis suggests a role for an intermediate ‘multilayered epithelium’, a structure composed of multiple layers of cells that appear squamous in their basal portion and columnar more superficially and express cytokeratins of both squamous and columnar differentiation [10].

## The stem cell theory
Increasingly interest is being generated by the hypothesis that Barrett’s oesophagus results from a change in the commitment of multipotent stem cells, which are induced to differentiate from a squamous into a columnar epithelium, as a result of the continuous exposure to environmental stresses such as refluxate. The stem cell theory would explain the variety of cellular phenotypes found in Barrett’s oesophagus, as well as how regeneration of squamous epithelium after removal of Barrett’s oesophagus is possible, and correlates well with evidence that the cell of origin is intrinsic to the oesophagus [9]. Furthermore, tissue-specific multipotent stem cells have been identified as key to unravelling the mechanisms involved in carcinogenesis [11]. In this regard, the most paradigmatic example is the recent identification of the stem cells in the intestinal epithelium [12] and the establishment of a link between the specific loss of the APC (adenomatous polyposis coli) gene and tumour development in the colon [13].

It should also be remembered that more than one population of progenitor cells could be present in the human oesophageal tissue, suggesting that the cellular origin of Barrett’s oesophagus could be multiple.

### Epithelial stem cells
The first stem cell population that could play a role in this theory are the epithelial cells residing in the basal layer of the squamous epithelium (Figure 1c). In the interfollicular epidermis, which is the most highly characterized squamous epithelium with features similar to the oesophagus, resident multipotent stem cells give rise to all the different cell lineages present within the adult tissue [14]. Previous label-retaining studies led to the conclusion that these slow but constantly dividing cells could generate early differentiated progeny (the so-called transit-amplifying cells) and therefore maintain the tissue homoeostasis and cell replacement necessary following injury [15,16]. However, a new emerging theory suggests that the role of the stem cells is merely to replenish cell loss following injury, whereas, in homoeostatic conditions, the sole cell progenitors responsible for the cell turnover are represented by a second self-sustaining population of early differentiated cells [17]. If this theory were proved to hold true outside the epidermis, the oesophageal...
epithelium and the development of OAC, through the metaplasia–dysplasia–neoplasia sequence, would represent an ideal model not only to understand tissue and cell response to injury, but also to track cell fate during carcinogenesis. Therefore, in order to investigate further the stem cell theory, identifying the epithelial stem cells of the squamous oesophagus has become essential; nevertheless, these cells have not been clearly detected and located.

Using animal models (mainly mouse), the homeostasis of the oesophageal epithelium has been successfully investigated, leading to the identification of a candidate stem cell population [18] in the basal layer of the oesophageal epithelium and the validation of CD34 as an epithelial stem cell marker [19]. However, the anatomy of the human oesophageal epithelium is remarkably different from that of the mouse, which is characterized by a less complex architecture; for example, it does not have papillae (finger-shaped structures of the lamina propria that invaginate into the epithelial basal layer, Figure 1), and it is constituted by far fewer cell layers. Therefore there are several limitations in the translation of this knowledge into humankind, which still remains an active area of debate.

In the early 1990s [20], a study comparing proliferating cell nuclear antigen staining and autoradiography to detect cell proliferation in oesophageal mucosa showed that the most slowly proliferating cells were found in the epithelial basal layer at the top of the papillae, suggesting that this could be the location of the stem cells. In contrast, more recently, work by Seery [21] and Seery and Watt [22] suggested that the multipotent stem cells resided in the inter-papillary basal layer of the oesophageal squamous epithelium, on the basis of their higher self-renewing potential, in comparison with those located in the papillary epithelium. In fact, following cell sorting based on the levels of β1-integrin expression and culture, the cells in the inter-papillary basal layer demonstrated a higher proliferation and clonogenic capability, in keeping with their potential ‘stemness’. However, these cells were identified and characterized by a very low expression of β1-integrin, which is at odds with previous findings that, instead, attributed a specific role to β1-integrin for the maintenance of the epithelial stem cell pool [23,24]. This work has shown that β1-integrin is highly expressed in undifferentiated cells and then lost when they commit to terminal differentiation [20–22]. These two studies [23,24] not only showed contradictory results, but also suffer from difficulties in correctly describing a highly irregular three-dimensional structure, such as the oesophageal epithelium, using two-dimensional models (paraffin-embedded or frozen tissue sections). Technological developments which allow for a three-dimensional representation of the epithelium using epithelial whole mounts and confocal microscopy are now being applied to revisit this question of stem cell location [25,26].

**Stem cells non-intrinsic to the epithelium**

A second population of stem cells may be located in the glandular neck region of the oesophageal submucosal gland ducts, similar to those found in the bulge region of hair follicles [27] (Figure 1d). Since these ducts are lined in their proximal two-thirds by columnar cells, whereas their distal third is lined with squamous cells [28], they have been suggested as a location for stem cells responsible for the origin of the columnar epithelium [29]. This hypothesis is based on the ulcer-associated cell lineage, that occurs adjacent to areas of ulceration in the gastrointestinal tract, and prefigures a migration of the glandular cells to the surface, through new glands and ducts generated by stem cells in the lamina propria [30]. The gland duct theory has recently been the subject of several studies which seem to support the concept that these calls may be critical for the development of Barrett’s oesophagus. First, RA (retinoic acid) has been identified as a stimulus for cell differentiation, suggesting that the stromal compartment, which includes the submucosal gland ducts, is the cell source for the columnar cells induced by RA treatment of squamous tissue [31]. Similarities between the submucosal glands and Barrett’s oesophagus have been highlighted by an immunohistochemical characterization study in pig tissues and cultures [32]. A histological study on serial sections of oesophageal resection tissue showed frequent gland ducts opening on to the surface of Barrett’s oesophagus epithelium [30]. In addition, a study on individual epithelial crypts has demonstrated that Barrett’s oesophagus heterogeneity arises from multiple independent clones. Furthermore, a p16 mutation was found in common between the submucosal gland duct and adjacent Barrett’s oesophagus epithelium, suggesting a common genetic origin for these cells [33]. Finally, in a similar study, islands of neo-squamous epithelium were found to be wild-type at loci containing mutations within the adjacent Barrett’s oesophagus epithelium. This suggests that the neo-squamous epithelium originates in different cells from those responsible for self-renewal of the Barrett’s oesophagus epithelium and gland ducts are a possible source [34]. More recently, it has been suggested that Barrett’s oesophagus could originate from bone-marrow-derived stem cells [35] and, although currently there are little data on this in the context of Barrett’s oesophagus, similar evidence has been found in gastric intestinal metaplasia [36].

**Conclusions**

The idea that reprogramming of stem cells plays a causal role in the pathogenesis of Barrett’s oesophagus is relatively new, interesting and supported by accumulating evidence. Many questions concerning the development of Barrett’s oesophagus, such as the location of these stem cells and the mono- or poly-clonal origin of the metaplastic epithelium, remain unanswered, but the detection and the characterization of the oesophageal progenitor cells would unveil crucial information in this regard and provide essential research tools, bearing in mind that more than one cell source could be involved.

**Funding**

This work is funded by the MRC-Cancer Cell Unit programme grant.
References


Received 24 September 2009
doi:10.1042/BSI20380370

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