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Mechanisms of Barrett's oesophagus: Intestinal differentiation, stem cells, and tissue models

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A B S T R A C T

Barrett's oesophagus (BE) is defined as any metaplastic columnar epithelium in the distal oesophagus which replaces normal squamous epithelium and which predisposes to cancer development. It is this second requirement, the predisposition to cancer, which makes this condition both clinically highly relevant and an important area for ongoing research. While BE has been defined pathologically since the 1950's (Allison and Johnstone, *Thorax* 1955), and identified as a risk factor for esophageal adenocarcinoma since the 1970's (Naef A.P., et al *J Thorac Cardiovasc Surg.* 1975), our understanding of the molecular events giving rise to this condition remains limited. Herein we will examine what is known about the intestinal features of BE and how well it recapitulates the intestinal epithelium, including stem identity and function. Finally, we will explore laboratory models of this condition presently in use and under development, to identify new insights they may provide into this important clinical condition.

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Introduction

Barrett's oesophagus (BE) occurs in the in the distal oesophagus in the setting of chronic gastro-esophageal reflux disease (GERD) and is histopathologically defined as the replacement of the normal squamous epithelium with an intestinalized columnar epithelium [1]. Clinically, the importance of Barrett's oesophagus lies in the observation that it is an important risk factor for esophageal adeno-carcinoma (EAC) [2]. This is not unique, as metaplasia has been linked to malignant transformation in other tissues including the stomach, where gastric intestinal metaplasia precedes gastric cancer, and in the cervix and lung, where squamous metaplasia in the columnar epithelium precedes the onset of cancer [3].

Efforts directed at understanding the pathogenesis of BE and its progression to EAC have been increasing over the last 15 years. Driving this increased focus has been the well-established observation that the rates of EAC have been increasing within the U.S. and western European populations over the last three decades [2]. While this imperative to better understand BE onset and progression to dysplasia and cancer has become clearer, much about the pathogenesis of this disease remains poorly understood. Herein we review the intestinal differentiation of BE and explore approaches currently used to study BE in the clinics and laboratories.

Molecular features of BE overlap with but do not completely recapitulate normal intestinal differentiation in the oesophagus

Histologically BE is characterized as the replacement of the normal multilayered squamous epithelium with a specialized columnar-lined epithelium containing intestinal-type goblet cells. In fact, there are three types of columnar epithelium observed in the distal oesophagus including [1] a junctional (cardia-type) epithelium [2], a gastric fundic-type epithelium with parietal and chief cells, and [3] a specialized, intestinal-type metaplasia with prominent goblet cells [4]. However, in a widely circulated position statement, the AGA defined Barrett's oesophagus as 'the condition in which any extent of metaplastic columnar epithelium that predisposes to cancer development replaces the stratified squamous epithelium … .' [5]. Therefore, it is this risk of progression to EAC that has led to the clinical focus on intestinalized metaplasia, although there are many questioning whether for clinical surveillance the focus should be broadened to include all columnar epithelium in the distal oesophagus.

BE is referred to as an 'intestinal' metaplasia because all four of the main intestinal epithelial cell lineages have been detected in BE tissues, including enterocytes, Paneth cells, enteroendocrine cells, and of course goblet cells. However, these cell lineages are not typically fully mature. At the ultra-structural level, enterocytes are frequently described as 'pseudoabsorptive' displaying apical microvilli, but mature cells with a well defined brush border are rare [6]. Nevertheless, these 'pseudoabsorptive' BE cells express many genes associated with absorptive enterocytes.

BE and associated tissues have been characterized using squamous-cell specific and glandular epithelial-cell specific cytokeratins (CK) and differentiation markers. CK7 and CK20 staining patterns may specifically distinguish BE from other histologically-related conditions including intestinal metaplasia of the gastric antrum and cardia [7,8]. Goblet cells are much more abundant in BE than in normal small intestine, and they are marked by the presence of acidic mucins detected by Alcian Blue staining, with *Mucin 2 (MUC2)* being perhaps the most important [9]. Like the cytokeratins, mucin expression patterns have been used to distinguish BE from cardia and antrum metaplasia, with *MUC1* and *MUC6* expression in BE distinct from mucin expression in the cardia and antrum [10].

Gene expression profiling revealed not only a similarity between normal upper GI (i.e. gastric and duodenal) mucosa and BE but stark differences between the squamous normal esophageal tissues and BE. Amongst genes relatively specific to BE are CK8, CK20, MUC2, MUC5AC, MUC6 and mucin-associated trefoil factors TFF1, TFF2 and TFF3 [11,12]. Analysing multiple datasets from six independent studies several transcription factors (CDX1, CDX2, HNF1, and HNF4) and the TGF-β/BMP pathway have been identified being significantly enriched in BE [13]. Homeobox transcription factors CDX1 and CDX2 are required for normal intestinal development, although their role in BE intestinal metaplasia and cancer is presently unclear. Both are induced in esophageal keratinocytes exposed to bile and acids