Best Practice & Research Clinical Gastroenterology 29 (2015) 113–124

Contents lists available at ScienceDirect

Best Practice & Research Clinical Gastroenterology

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The use of molecular markers in predicting dysplasia and guiding treatment

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Keywords:
Barrett's oesophagus
Molecular markers of cancer
Oesophageal adenocarcinoma
Barrett's endoscopy

A B S T R A C T

The ability to stratify patients based on the risk of progression to oesophageal adenocarcinoma would provide benefit to patients as well as deliver a more cost effective surveillance programme. Current practice is to survey all patients with Barrett's oesophagus (BO) and use histological diagnoses to guide further management. However, reliance on histology alone has its drawbacks. We are currently unable to reliably stratify the risk of progression of patients with non-dysplastic BO based on any particular histological feature. There is also considerable variability in histological interpretation. An obvious recourse has been to rely on identifying molecular features possibly as an adjunct to histology, to better diagnose and stratify patients. To this end, p53 immunohistochemistry can be used as a useful adjunct to risk stratify and clarify histological grades, particularly low-grade dysplasia. Other markers of progression, although not yet in a clinically applicable format, are promising. Measurements of promoter methylation and also genomic instability such as loss of heterozygosity and copy number alterations show promise especially as high throughput genetic technologies reach maturity. The enduring hope is that these molecular biomarkers will make the transition to clinical applicability either in the direct endoscopic setting or even using non-endoscopic methods.

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http://dx.doi.org/10.1016/j.bpg.2014.11.003
1521-6918/

Introduction

Epidemiologically, Barrett's oesophagus (BO) remains a difficult problem. The overall risk of a patient with BO developing an oesophageal adenocarcinoma (OAC) remains small at approximately 0.3% [1] and this statistic is often used in arguments to justify not surveying this population. However the converse to this argument is that the risk of developing OAC in a patient with BO is 50 times that of the general population. The argument for non-surveillance is similarly strengthened by economic modelling of the cost of surveillance when implemented as a national surveillance programme [2] as it is in several countries [3,4]. Again, however, such surveillance programmes clearly detect a potentially incurable cancer at a stage where it is curable and often without the need for major surgery or chemotherapy [5]. The balance between arguments of the cost of national surveillance and the fact that the programmes detect cancers at an earlier and more curable time-point is rendered difficult because the epidemiology is largely based on an overall BO population prevalence whereas it is becoming increasingly clear that not all BO has the same risk of progression. A major undertaking therefore in BO research has been in defining markers, molecular and otherwise, of progression so that patients can be risk stratified according to the likelihood of progression. However, the biology of BO has proved difficult in no small part because the tissue is non-uniform and *protean* [6]. The stem cell compartment (often presumed to be the origin of carcinogenic clones) of the normal oesophagus and of BO has still not been determined and in fact what has been often assumed to be a metaplasia may not be at all [6]. Initial attempts at determining risk were therefore often based on molecular pathways known to be errant in other tissues.

The progression of BO from a columnar lined oesophagus with or without goblet cells, to adenocarcinoma is a well established and a probably linear progression from non-dysplastic BO (NDBO) to low-grade dysplasia (LGD) and high grade dysplasia (HGD) before the development of oesophageal cancer (OAC). The histopathological grade is the current gold standard to determine the overall risk of progression. LGD is also difficult to define with original studies demonstrating low inter-observer agreement with κ values of 0.18 and 0.35 [7] and frequent over diagnosis [8] which may have been responsible for the predicted varying risk of malignant progression ranging from 0.6% to 13.4% [9–11]. Subsequent analyses considering only samples with expert consensus for LGD have substantially upgraded the risk of malignant progression if LGD is present [12]. There is therefore a clear need to determine further markers of progression from non-dysplastic BO. This is all the more necessary given the introduction of endoscopic ablation therapies now available for all grades of dysplasia [13,14].

P53

Table 1 Because of its technical maturity and the ability to directly visualise stains as applied to intact histological morphology, immunohistochemistry (IHC) has been widely studied in BO progression. P53 is expressed from the gene *TP53* (chromosome 17p). It is a major tumour suppressor gene and is the most commonly mutated gene in human cancers [15]. The main functions of p53 are to activate the DNA repair mechanisms if damage has occurred, activate p21 mediated cell cycle arrest at the G1/S cell cycle checkpoint, and to initiate apoptosis if DNA damage cannot be repaired [16]. The p53 protein contains five major domains. The N-terminal transcription-activation domain (TAD) that activates transcription factors; a proline-rich domain that allows interactions with other proteins; a DNA binding domain (exons five to eight); a tetramerization domain crucial for p53 activity *in vivo* and a regulatory C terminus domain. P53 is constantly produced by every cell yet in the absence of DNA damage, murine double minute two protein (mdm2) monoubiquitinates p53 and thus it is degraded [17]. P53 accumulation occurs when conformational changes initiated by DNA damage or stress, prevent the mdm2-P53 interaction and thus prolong its half-life from minutes to hours.

It is also assumed that the gene *TP53* is abnormal in areas where p53 IHC demonstrates an abnormal stain. In BO progression, its function is most often altered or lost by either mutation or loss of heterozgosity (LOH). Mutations most commonly occur in the DNA binding domain of the gene and can result in conformational changes that prevent ubiquitination and removal from the tissue. The resultant stabilization of the protein results in its increased nuclear intensity on immunohistochemistry. *TP53* mutations are uncommon in non-dysplastic BO mucosa [18,19] (but possibly more common if the non-