Review Article

Biomarkers in Barrett's oesophagus

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Abstract: Barrett's oesophagus (BO) is the most important risk factor for oesophageal adenocarcinoma (OAC) and its incidence, as well as the cancer it precedes, is increasing. Evidence suggests that current methods of screening and surveillance are inadequate and therefore more clinically and cost effective techniques have been sought. With a better understanding of oesophageal adenocarcinoma and cancer in general, several genetic biomarkers have been identified which could be used to better diagnose and risk stratify patients. In this article we review current evidence for biomarkers in BO as well as identifying some of the problems yet to be overcome to allow their transition into clinical practice.

Keywords: Barrett's oesophagus (BO); oesophageal adenocarcinoma; biomarker

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Introduction

Barrett's oesophagus (BO) is the most important risk factor for oesophageal adenocarcinoma (OAC). Although the vast majority of patients with the condition will not develop cancer, the implications for those that do are grave if not detected early. In order to identify this pre-malignant change as early as possible, strategies of endoscopic screening and surveillance in patients at highest risk are employed across most Western countries. However, this may be exposing large numbers to repeated and unnecessary procedures, putting both patients and resources at risk. Establishing ways to identify which patients require and do not require continued surveillance has been at the centre of much recent research. In particular, the discovery of new molecular biomarkers in OAC has provided an important focus in the attempt to better risk stratify patients. Further studies to evaluate better targeting of at-risk patients are underway and together with novel cell sample retrieval methods, the utilisation of molecular biomarkers within a surveillance program is likely to play an important role in improving outcomes for patients with BO in the future. This article will review current evidence in biomarkers in BO and summarise the problems still to be overcome to allow their transition into clinical practice.

Current screening and surveillance in BO

BO is defined as the metaplasia of normal stratified squamous epithelium of the distal oesophagus to columnar epithelium. This pathological change is likely to be driven by persistent gastroesophageal reflux disease (GORD) however there are a number of important risk factors for its development. Since the 1990s the incidence of BO, as well as the cancer it precedes, has risen across the Western world (1). Although the estimates vary, BO is likely to affect between 1% and 6% of the general population (2-4). Unless detected early, mortality rate from OAC remains over 80% at 5 years. However, the progression of BO into cancer is not inevitable. In fact, conversion rate to OAC is only around 0.33% per year indicating that a significant proportion of those with BO will not develop cancer (5).

At worst, BO demonstrates a stepwise progression from non-dysplasia, to low or high-grade dysplasia, into adenocarcinoma. At best, BO may lie dormant and asymptomatic for life. Identifying which patients fall into each group would be a significant step towards a more efficient surveillance strategy.

Currently, BO screening In the UK and USA is reserved for those patients with symptoms of chronic GORD and specific risk factors. It is diagnosed within screening programs or incidentally by endoscopic evidence of columnar lined epithelium according to Prague criteria and histopathological analysis. For patients in whom BO is diagnosed, the treatment or surveillance strategy is decided upon based on size of Barrett's segment and certain histopathological features, the assessment of which are both relatively subjective. Important histopathological features include grade of dysplasia, which if present could be high or low and confers a higher risk of progression to cancer, and the presence of intestinal metaplasia which is currently not a prerequisite for diagnosis of BO in the UK, as it is in the USA, and also is likely to confer a higher risk of progression. As previously discussed, the conversion of BO to malignancy is relatively low compared to other cancers and this is particularly true of non-dysplastic BO which is estimated to have a conversion rate of between 0.12% and 0.16% per year (6). Repeat surveillance endoscopy occurs between 2 and 5 years depending on the above factors, however, given low conversion rate from BO to cancer, suitability of surveillance programs in their current form have been cast in some doubt. Although there has been some evidence of a positive impact on survival (7), some studies argue against the need for surveillance (8), whilst others conclude that the current approach to early detection of OAC has little effect on mortality (9). Therefore, studies to develop more effective surveillance strategies are underway with new biomarkers for BO considered a possible candidate incorporation into updated screening programs.

Candidates for biomarkers in BO

Over the past two to three decades our understanding of cancer and how it evolves has advanced significantly. Cancer cells multiply and evade destruction by gaining a competitive advantage usually conferred by genetic mutations. Pre-malignant tissues such as BO are a product of these changes and are a platform from which tumours can develop. Epithelial cells of the oesophagus develop into OAC gradually, and via a well-established sequence of metaplasia and dysplasia (10). This linear progression is common across a number of cancers including colon, breast, prostate, cervical and bladder. The rate at which these cancers develop from their precursor lesions is variable, with time to cancer spanning years to decades. Therefore, the detection of precursor lesions provides an ideal opportunity to diagnose and treat the patient before cancer develops. This is the basis of a number of successful screening programs including those for bowel and gastric cancer. However, as previously discussed, the introduction of current surveillance programs for BO and OAC has had little effect on mortality (9). In order to develop better surveillance programs for cancer and OAC in particular, there has been a growing interest in understanding changes within cancer cells at a molecular level. Understanding and identifying these changes could promote more focused cancer prevention strategies, reducing the problems of over diagnosis in mass screening populations. Therefore, biomarkers in BO play two distinct roles; firstly, to aid diagnosis and potentially reduce the number of patients required to undertake endoscopy; and secondly, to monitor patients and more accurately assess the risk of developing OAC.

Biomarkers for screening

BO is usually diagnosed via endoscopic and histopathological analysis of oesophageal epithelial cells. However, advancing technologies including gene expression analysis, epigenetics, proteomics and single nucleotide polymorphism (SNP)-based platforms have provided more opportunities for biomarker discovery in both BO and OAC (11). The most comprehensively studied biomarker for screening of BO is Trefoil factor 3 (TFF3). Trials to evaluate TFF3 as an immunohistochemically identifiable biomarker have taken place using a minimally invasive sampling device called the Cytosponge (12-14). The device, which is swallowed by the patient and retrieves cells from the distal oesophagus whilst being pulled back using a string, has been shown to be easy to use, acceptable to patients and could be for in a community setting. Using the TFF3 as a biomarker, analysis of the retrieved sample provides an objective, binary read out of the presence or absence of BO (13). Studies have so far demonstrated a sensitivity of around 80%, increasing to 87% for patients with over 3cm of circumferential BO. In patients who swallowed the device twice, sensitivity increased to around 90% whilst sensitivity was demonstrated to be approximately 92%. Utilising the same technology,

other biomarkers and potential targets for BO screening have been proposed by the same research group. In a 2017 study by Chettouh *et al.* up to 18 genes were found to be mutated in BO, four of which (TFPI2, TWIST1, ZNF345 and ZNF569) were differentially methylated to normal squamous and gastric cardia tissues (14). This new technology, in combination with novel genetic changes in BO technique, seems to provide a viable alternative to traditional endoscopic methods. This approach may be able to reach a much wider group of patients with gastro oesophageal reflux disease at risk of developing BO and OAC (15).

Biomarkers for surveillance

Clinical biomarkers are an extremely useful tool in characterising and differentiating cell tissues. Importantly, they can be measured objectively, eliminating interpretation error and providing a more reliable base for clinical decision making. As described above, the identification of dysplasia in oesophageal epithelial cells is subjective. A more desirable biomarker would identify development earlier in the cancer sequence and would be a more objective measure of progression. However, despite the knowledge of thousands of biomarkers for a range of cancers, transitioning these discoveries into clinical practice has proven difficult. Current evidence suggests that there is extensive genetic change in the majority of advanced cancers (16).

Tissues which demonstrate BO and OAC are no exception and data from high density SNP arrays and exon-sequencing studies suggest a wide range of mutations in number different genes (17,18). Genes of oesophageal epithelial cells may be inherently damaged or mutated by a number of different mechanisms. DNA content abnormalities and loss of heterozygosity (LOH) such as aneuploidy and tetraploidy are a well-established feature of cancer cell biology and also occur in BO and OAC, resulting in mutations which confer inactivation of the tumour suppressor gene p53 (19). Mutations within another tumour suppressor gene, p16, have also been found to be one of the earliest changes in BO, and result in clonal expansion (20). However, p16 is unlikely to be a suitable candidate for a biomarker given that it appears early in the development of cancer and has been not been shown to be associated with grade of dysplasia (11,21). Other interesting biomarkers for BO include the cell cycle markers Cyclin A and D, which when present, indicate inactivation of p105-Rb. In particular, in one study presence of Cyclin D indicated that patients with BO were more likely to progress to OAC. However, these findings were not replicated in a larger, population-based study (22). Interestingly, a significant number of these mutations identified are found in adjacent BO and OAC tissue (within the same patient), suggesting that genetic changes maybe detectable within individuals who are risk of developing OAC prior to the malignant change. However, there are a large number of mutations which seem to occur independently of disease stage.

Further assessment for genetic diversity was undertaken in a 2016 by Martinez *et al.* in study of clonal evolution in non-dysplastic BO (23). Using fluorescence *in situ* hybridization techniques (FISH), a method in which labelled DNA probes are used for detection of chromosomal and specific gene aberrations, clonal mosaicism itself was found to be a powerful predictor of cancer development. Specifically, single-probe diversity measures (MYC and CEP 7) were identified as best predictors of progression, whereas p16 abnormalities were least predictive due to significant expansion and contraction across samples. The study remained consistent with previous studies which found degree of genetic divergence remain consistent between 'non-progressors', in contrast to 'progressors' which demonstrated significant genetic diversity 24–48 months prior to progression (24). Previous studies using FISH have also detected aneuploidy in chromosomes 7 and 17, including within p53, correlating it with the progression of both IM to LGD and from LGD to HGD (25,26).

An important 2014 study by Weaver *et al.* found common mutations across BO, HGD and OAC samples included ARID1A and SMARCA4, members of the SWI/SNF complex (27). However, these mutations also occurred in 'never-dysplastic' samples and have unclear significance in the development of OAC. In the same study, of other common mutations which included ABCB1, CNTNAP5, MYO18B, TP53 and SMAD4, only the latter two were found to confer a risk of cancer development. However, although the presence of SMAD4 clearly demonstrated risk of progression to cancer, it was found at a relatively low frequency within OAC tissue (13%). Furthermore, it was most effective at distinguishing between HGD and OAC, a point of distinction which matters less in terms of the therapeutic interventions. In this study, TP53 was found to be mutated in both HGD (72%) and OAC (69%) samples, but only 1 case (2.5%) of 'never-dysplastic' oesophagus. This builds on previous work which has demonstrated a significant increase in progression to OAC in those samples containing defects within the TP53 gene. In combination

with another LOH mutation on chromosome 9p, a 'panel', in combination with p53 protein provided the best predictor of BO to OAC to date (11). Whilst promising in its own right, this also suggests that 'biomarker panels' containing numerous foci of mutations may provide the most comprehensive assessment of risk stratification.

The future of biomarkers: application into clinical practice

In order for screening and surveillance programs for BO to be improved, intervention must become more targeted. Biomarkers clearly have potential to provide this focus but given their heterogeneity in the genetic patient profiles of those progressing to cancer, identifying very low risk patients may be a more achievable strategy (28,29). Identifying those at low risk would enable better risk stratification of patients which could direct resources to patients who need treatment most as well as spare those at low cancer risk of unnecessary endoscopy. Given the disappointing effect screening and surveillance in BO and OAC has demonstrated in some early studies, new technology such as the Cytosponge could be an important addition to the field. As previously discussed, this technique has the potential to reach a significant number of patients who might otherwise evade detection and is currently the subject of a 9,000-patient primary care study called 'BEST3' being undertaken in the UK. As well as use in screening for BO, risk stratification for malignant potential could be performed using additional biomarkers on the same Cytosponge sample following evaluation for the BO, providing additional information on likelihood of progression to cancer and therefore more suitable screening intervals. In terms effects on treatment, continued screening and biomarker characterization may lead to Identification of a biological basis of recurrence of BO and OAC after ablation or even immunotherapy, enabling targeting of specific genetic mutations although much more research is required to elucidate potential targets (29).

Conclusions

Understanding the likelihood of progression to cancer of tissues with certain molecular features could allow clinicians to more easily decide when to treat patients and with which therapy. Importantly, they could also help to identify those who do not need treatment at all, and instead, aid risk stratification to develop more personalised surveillance intervals. The accumulation of genetic mutations in a stepwise manner is a feature of all cancers, however, the number and variability of changes in BO and OAC is particularly high. The most promising data suggests that using biomarkers for BO are most effective within 'biomarker panels'. In combination with more focussed targets for screening in BO it seems highly likely that screening for BO, within the context of a mass screening program, will become more clinically and cost effective as well as less invasive. We are someway off a comprehensive understanding of what drives oncogenic change in OAC and furthermore, delivering routine tests on the scale required may still prove logistically and financially difficult. However, in light of recent evidence, it seems promising that the identified biomarkers will be developed and new ones will be found, which when used in combination, could signal radical improvement on our current screening and surveillance programs.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jlpm.2018.10.02). The authors have no conflicts of interest to declare.

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References

1. Kadri SR, Lao-Sirieix P, O'Donovan M, et al. Acceptability

and accuracy of a non-endoscopic screening test for Barrett's oesophagus in primary care: cohort study. BMJ 2010;10:341:c4372.
2. Ronkainen J, Aro P, Storskrubb T, et al. Prevalence of Barrett's esophagus in the general population: an endoscopic study. Gastroenterology 2005;129:1825-31.
3. Zagari RM, Fuccio L, Wallander MA, et al. Gastro-oesophageal reflux symptoms, oesophagitis and Barrett's oesophagus in the general population: Loiano-Monghidoro study. Gut 2008;57:1354-9.
4. Hayeck TJ, Kong CY, Spechler SJ, et al. The prevalence of Barrett's esophagus in the US: estimates from a simulation model confirmed by SEER data. Dis Esophagus 2010;23:451-7.
5. Desai TK, Krishnan K, Samala N, et al. The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. Gut 2012;61:970-6.
6. Hvid-Jensen F, Pedersen L, Drewes AM, et al. Incidence of adenocarcinoma among patients with Barrett's esophagus. N Engl J Med 2011; 365:1375-83.
7. Rubenstein JH, Sonnenberg A, Davis J, et al. Effect of a prior endoscopy on outcomes of esophageal adenocarcinoma among United States veterans. Gastrointest Endosc 2008;68:849-55.
8. Corley DA, Mehtani K, Quesenberry C, et al. Impact of endoscopic surveillance on mortality from Barrett's esophagus–associated esophageal adenocarcinomas. Gastroenterology 2013;145:312-9.e1.
9. Reid BJ. Genomics, endoscopy, and control of gastroesophageal cancers: a perspective. Cell Mol Gastroenterol Hepatol 2017;3:359-66.
10. Zeki S, Fitzgerald RC. Targeting care in Barrett's oesophagus. Clin Med (Lond) 2014;14:s78-83.
11. Ong CA, Lao-Sirieix P, Fitzgerald RC. Biomarkers in Barrett's esophagus and esophageal adenocarcinoma: predictors of progression and prognosis. World J Gastroenterol 2010;16:5669-81.
12. Fitzgerald RC, Reid BJ. Early diagnosis of gastroesophageal cancers and the cytosponge: a work in progress. Cell Mol Gastroenterol Hepatol 2017;4:447.
13. Ross-Innes CS, Debiram-Beecham I, O'Donovan M, et al. Evaluation of a minimally invasive cell sampling device coupled with assessment of trefoil factor 3 expression for diagnosing Barrett's esophagus: a multi-center case–control study. PLoS Med 2015;12:e1001780.
14. Chettouh H, Mowforth O, Galeano-Dalmau N, et al. Methylation panel is a diagnostic biomarker for Barrett's oesophagus in endoscopic biopsies and non-endoscopic cytology specimens. Gut 2018;67:1942-9.
15. Riley T, Ang Y. Methylation panel as a diagnostic biomarker in Barrett's oesophagus: a comprehensive biomarker panel in a population-based screening programme? J Lab Precis Med 2018;3:37.
16. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012;366:883-92.
17. Dulak AM, Schumacher S, van Lieshout J, et al. Gastrointestinal adenocarcinomas of the esophagus, stomach and colon exhibit distinct patterns of genome instability and oncogenesis. Cancer Res 2012;72:4383-93.
18. Dulak AM, Stojanov P, Peng S, et al. Exome and whole-genome sequencing of esophageal adenocarcinoma identifies recurrent driver events and mutational complexity. Nat Genet 2013;45:478-86.
19. Reid BJ, Levine DS, Longton G, et al. Predictors of progression to cancer in Barrett's esophagus: baseline histology and flow cytometry identify low-and high-risk patient subsets. Am J Gastroenterol 2000;95:1669-76.
20. Galipeau PC, Cowan DS, Sanchez CA, et al. 17p (p53) allelic losses, 4N (G2/tetraploid) populations, and progression to aneuploidy in Barrett's esophagus. Proc Natl Acad Sci 1996;93:7081-4.
21. Wong DJ, Paulson TG, Prevo LJ, et al. p16INK4a lesions are common, early abnormalities that undergo clonal expansion in Barrett's metaplastic epithelium. Cancer Res 2001;61:8284-9.
22. Murray L, Sedo A, Scott M, et al. TP53 and progression from Barrett's metaplasia to oesophageal adenocarcinoma in a UK population cohort. Gut 2006;55:1390-7.
23. Martinez P, Timmer MR, Lau CT, et al. Dynamic clonal equilibrium and predetermined cancer risk in Barrett's oesophagus. Nat Commun 2016;7:12158.
24. Li X, Galipeau PC, Paulson TG, et al. Temporal and spatial evolution of somatic chromosomal alterations: a case-cohort study of Barrett's esophagus. Cancer Prev Res (Phila) 2014;7:114-27.
25. Timmer MR, Sun G, Gorospe EC, et al. Predictive biomarkers for Barrett's esophagus: so near and yet so far. Dis Esophagus 2013;26:574-81.
26. Pacha A, Rygiel AM, Westra W, et al. Su1181 A diagnostic DNA fish biomarker assay identifies HGD or EAC in Barrett esophagus. Gastroenterol 2012;142:S-445.
27. Weaver JM, Ross-Innes CS, Shannon N, et al. Ordering of mutations in preinvasive disease stages of esophageal carcinogenesis. Nat Genet 2014;46:837-43.
28. Ross-Innes CS, Chettouh H, Achilleos A, et al. Risk stratification of Barrett's oesophagus using a non-

endoscopic sampling method coupled with a biomarker panel: a cohort study. Lancet Gastroenterol Hepatol 2017;2:23-31.
29. Secrier M, Li X, De Silva N, et al. Mutational signatures in esophageal adenocarcinoma define etiologically distinct subgroups with therapeutic relevance. Nat Genet 2016;48:1131-41.

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