BMJ Open

Epigenetic biomarkers in progression from non-dysplastic Barrett's oesophagus to oesophageal adenocarcinoma: a systematic review protocol

T Nieto,[1] C L Tomlinson,[2] J Dretzke,[3] S Bayliss,[3] M Dilworth,[4] A D Beggs,[1] O Tucker[1,4]

To cite: Nieto T, Tomlinson CL, Dretzke J, *et al*. Epigenetic biomarkers in progression from non-dysplastic Barrett's oesophagus to oesophageal adenocarcinoma: a systematic review protocol. *BMJ Open* 2016;**6**:e013361. doi:10.1136/bmjopen-2016-013361

► Prepublication history and additional material is available. To view please visit the journal (http://dx.doi.org/10.1136/bmjopen-2016-013361).

Received 26 July 2016
Revised 21 October 2016
Accepted 10 November 2016

For numbered affiliations see end of article.

Correspondence to
T Nieto;
t.nieto@bham.ac.uk

ABSTRACT

Introduction: Barrett's oesophagus (BO), a metaplastic condition affecting the lower oesophagus due to long-standing gastro-oesophageal reflux and chronic inflammation, is a precursor lesion for oesophageal adenocarcinoma (OADC). There is no clinical test to predict which patients with BO will progress to OADC. The British Society of Gastroenterology recommends endoscopic surveillance of patients with BO. Epigenetic changes have been well characterised in the neoplastic progression of ulcerative colitis to colonic carcinoma, another gastrointestinal cancer associated with chronic inflammation. This systematic review protocol aims to identify and evaluate studies which examine epigenetic biomarkers in BO and their association with progression to OADC.

Methods and analysis: All prospective and retrospective primary studies, and existing systematic reviews investigating epigenetic markers including DNA methylation, histone modification, chromatin remodelling, micro and non-coding RNAs of all types will be eligible for inclusion. Eligible patients are those over the age of 18 with BO, BO with dysplasia, OADC or unspecified oesophageal cancer. A comprehensive search of bibliographic databases using combinations of text and index words relating to the population, prognostic markers and outcome will be undertaken with no language restrictions. Results will be screened by 2 independent reviewers and data extracted using a standardised proforma. The quality and risk of bias of individual studies will be assessed using the Quality in Prognostic Studies (QUIPS) tool. A narrative synthesis of all evidence will be performed with key findings tabulated. Meta-analysis will be considered where studies and reported outcomes are considered sufficiently homogeneous, both clinically and methodologically. Findings will be interpreted in the context of the quality of included studies. The systematic review will be reported according to PRISMA guidelines.

Ethics and dissemination: This is a systematic review of completed studies and no ethical approval is required. Findings from the full systematic review will be submitted for publication and presentation at national and international conferences which will inform future research on risk stratification in patients with BO.

Review registration number: CRD42016038654.

Strengths and limitations of this study

- Systematic review protocol following PRISMA-P guidelines, including description of key methodological steps.
- Rationale for a new systematic review in this area based on scoping searches.
- Exhaustive search strategy likely to capture all relevant published literature on epigenetic markers for progression of Barrett's oesophagus and oesophageal adenocarcinoma.
- Heterogeneity of published research anticipated (differing epigenetic biomarkers studied, variation of study design, sampling methods and follow-up length).
- Above may limit certain epigenetic markers to narrative evidence synthesis

INTRODUCTION

The incidence of oesophageal adenocarcinoma (OADC) has dramatically increased in recent years to 5.7 per 100 000 for women and 14.1 per 100 000 for men in the UK.[1 2] Unfortunately the majority of patients present with advanced unresectable disease with an overall 5-year survival of <13%.[3] The five-year survival rates improve considerably to 39% with localised disease.[4] Barrett's oesophagus (BO), is defined as an oesophagus in which any portion of the normal distal squamous epithelial lining is replaced by metaplastic columnar epithelium which is

clearly visible endoscopically (≥1 cm) above the gastro-oesophageal junction (GOJ) and confirmed histopathologically from oesophageal biopsies.[5] BO arises due to long-standing gastro-oesophageal reflux disease (GORD) and chronic inflammation and is a precursor lesion for OADC with progression through the metaplasia-dysplasia-carcinoma sequence.[6] The likelihood of developing OADC is increased 1.7 times in patients with GORD, increasing to 10.6 times with BO.[7] The incidence of OADC has risen in parallel with increasing obesity and GORD in Western populations.[1] With rising rates of obesity the incidence of OADC is predicted to further increase.[8] Currently there is no robust way of predicting which patients with BO will progress to OADC. The current clinical biomarker for the progression of BO is the presence of worsening cellular dysplasia, also known as intraepithelial neoplasia, on histological examination of serial oesophageal biopsies.[5] The presence of high-grade dysplasia (HGD), and recently low-grade dysplasia (LGD), triggers intervention.[9] As a result, the British Society of Gastroenterology recommends endoscopic surveillance of patients with BO and the American College of Gastroenterology endorses screening of high-risk patients for BO.[5 10] Endoscopic surveillance is invasive, expensive and despite rigorous biopsy protocols, dysplasia and early cancers can be missed. Importantly a meta-analysis published in 2012 demonstrated lower risk for progression of non-dysplastic BO than previously reported with a pooled 0.33% (95% CI 0.28% to 0.38%) annual incidence of OADC.[11] The annual incidence rate of OADC for patients with BO with HGD is 7–19%.[12–14]

Epigenetics is an emerging field which describes mechanisms of alteration of gene regulation and expression without changing the genetic code.[15] These regulatory mechanisms are important in normal human development, for example, silencing of the X-chromosome in females.[16] Epigenetic changes may be inherited but can also be acquired through environmental factors such as cigarette smoking.[17] Epigenetic change can occur through various methods. The most recognised are covalent modifications including DNA methylation, histone modification and altered gene expression by non-coding RNAs.[15] DNA methylation occurs when DNA methyltransferase adds a methyl group (CH_3) to a DNA base. In humans this is most commonly a cytosine base creating 5-methylcytosine.[15] Methylation, which occurs at gene promoter (CpG) sites causes downregulation of these genes. It is thought that the mechanism responsible is the projection of a methyl group into the DNA groove which physically blocks transcription.[18] Histone modification is a post-translational alteration to histone proteins which package DNA into nucleosomes and eventually chromosomes by winding DNA around them. If the histone structure is altered, the DNA cannot be correctly unravelled and cannot be correctly transcribed. The above modifications are carried over when a cell divides and can be inherited.[19] Many different types of non-coding RNAs have been discovered to alter gene expression by targeting coding messenger RNA (mRNA) after its transcription from DNA. Both micro RNAs (miRNA) and long non-coding RNAs have been implicated in gene regulation. These bind to mRNA molecules and cause them to be denatured and halt protein translation and cause genetic silencing.[20]

Abnormal silencing of a tumour suppressor or a DNA repair gene through hypermethylation of their CpG promoter sites may cause the cells to grow uncontrollably and lead to tumourigenesis. Epigenetic changes have been well characterised in the neoplastic progression of ulcerative colitis,[21–24] another tumour arising as a result of chronic inflammation progressing through dysplasia and resulting in colonic carcinoma.[25] Intriguingly epigenetic change has been shown to occur early in this process before neoplasia has developed.[26] The Enhanced Neoplasia Detection and Cancer Prevention in Chronic Colitis (ENDCaP-C) trial is investigating whether a panel of methylated biomarkers detected in endoscopic biopsy samples can be used as a tool in conjunction with screening colonoscopy to help risk stratify patients who are at higher risk of progressing to carcinoma.[27] With the latest next generation sequencing and methylation microchip arrays, it is possible to detect epigenetic changes accurately and reproducibly even in archival tissue samples. In light of this there is a need to consolidate the literature on epigenetic changes in Barrett's carcinogenesis to determine if such changes provide a method of risk stratifying patients who are at risk of progression to OADC.

A scoping search was performed using MEDLINE, the Cochrane Library and internet sources to identify any systematic reviews or meta-analyses on epigenetic biomarkers in BO and oesophageal cancer (OC). It revealed in excess of 2000 primary studies which are relevant for inclusion into the proposed systematic review. No systematic reviews which draw together all aspects of epigenetic change within the field of Barrett's carcinogenesis were identified. Nine systematic reviews and meta-analyses were identified[28–36] which included mixed patient populations with OADC and oesophageal squamous cell carcinoma (OSCC) with only three reviews incorporating patients with BO.[31 32 36] These studies concentrated on a single type of epigenetic alteration with four investigating DNA methylation[28–30] and three looking at miRNA expression.[31–34] The remaining two studies investigated genetic alterations in progression of BO to OADC.[35 36] Based on these results, we believe that a systematic review on this topic is both timely and required.

Research aims

This systematic review will identify and summarise studies which examine epigenetic biomarkers in BO and their association with progression to OADC with the aim of consolidating the literature and informing future laboratory work.

METHODS AND ANALYSIS

This systematic review protocol has been reported in accordance with PRISMA-P guidelines.

Selection criteria

Population: All patients over the age of 18 with BO, BO with dysplasia, OADC or unspecified OC will be included.

Prognostic markers: Epigenetic markers including DNA methylation, histone modification, chromatin remodelling, miRNAs and non-coding RNAs of all types will be included.

Outcome: Progression from non-dysplastic BO with or without intestinal metaplasia to BO with LGD, HGD or OADC.

Study design: All prospective and retrospective primary studies, and systematic reviews will be included.

Publication type: Abstract and full texts will be included with exclusion of letters and editorials

Exclusion criteria: OSCC and established OCs with no evidence of a pre-existing BO diagnosis will be excluded. Case reports, narrative reviews, in vitro studies (eg, cell lines), studies of genetic mutations, studies using biomarkers to predict a response to treatment (eg, chemotherapy) will be excluded. A decision was made to exclude animal studies, as scoping searches indicated that there were comparatively few (compared with human studies), and therefore were likely to add heterogeneity to an already heterogeneous evidence base. In addition, we concluded that issues relating to transferability of experimental findings from animal models to a clinical setting would occur.

Search strategy

The following electronic bibliographic databases will be searched from inception: EMBASE, MEDLINE, MEDLINE in Process, DARE, CDSR, Cochrane Central. Conference (Conference Proceeding Citation Index, Zetoc) and registers of clinical trials (ClinicalTrials.gov and ICTRP) will also be searched. Reference lists of identified studies and systematic reviews will be screened for any relevant primary studies that were not retrieved from the database searches. Date or language restrictions will not be placed on searches. A search strategy will be developed using combinations of text and index words relating to the population, exposure and outcome, such as: 'Barrett's Oesophagus', 'epigenetic', 'DNA methylation', 'marker' and 'oesophageal adenocarcinoma'. A sample search strategy for MEDLINE is shown in online supplementary appendix 1.

Study selection

This will be a two-step process. Titles and abstracts identified in our literature search will be screened independently by two reviewers using prespecified screening criteria. These are broadly based on whether the studies first include measuring epigenetic markers in patients with OADC and second whether these patients have progressed from BO to OADC. Full texts of any potentially relevant articles will be obtained and subjected to the full inclusion criteria. Any discrepancies found will be referred to a third reviewer. The study selection process will be documented using the PRISMA flow diagram. Endnote X7 will be used as reference management software and decisions on inclusion or exclusion will be recorded.

Data extraction

Data will be extracted by two independent reviewers using an agreed, standard data extraction form. Any disagreements which cannot be resolved by discussion will be referred to a third reviewer who will act as an arbitrator.

Data will be extracted on the following study characteristics:

1. Study design characteristics—for example, prospective or retrospective and length of follow-up.
2. Population—for example, tissue samples from patients with BO or patients with OADC looking retrospectively at BO samples, patient demographics.
3. Prognostic markers—epigenetic markers including DNA methylation, histone modification, chromatin remodelling, miRNAs and non-coding RNAs of all types.
4. Outcomes—progression from non-dysplastic BO with or without intestinal metaplasia to BO with LGD, HGD or OADC.

Assessment of study quality

The quality and risk of bias of individual studies will be assessed using the Quality in Prognostic Studies (QUIPS) tool.[37] This tool will review each individual study in six criteria: study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding factors, and statistical analysis and reporting. We anticipate that due to the difficulty in obtaining samples and the length of follow-up required to assess progression from BO to OADC, there may be significant sample selection bias. Eligible studies are likely to be subject to confounding, with main confounding factors relating to age, obesity, smoking and alcohol intake. The risk of bias assessment will therefore include an assessment of which confounding factors (if any) have been measured and whether they were adjusted for in the design or analysis of the study. There may be differences in how robust the methods are for measuring the prognostic markers and the outcome; for example, published guidelines recommend confirmation of HGD by two independent pathologists.[5] These factors need to be assessed carefully for each study so that a judgement can be made on whether epigenetic changes seen in these studies are truly reflective of Barrett's carcinogenesis on a population level and whether they can be reproduced easily and accurately for screening purposes. We do not anticipate finding any studies that test models

predicting progression based on patient factors and panels of epigenetic markers.

Evidence synthesis

A narrative synthesis of all evidence will be performed with key findings tabulated. An assessment of clinical and methodological heterogeneity will be undertaken in order to determine the feasibility of meta-analysis. The main sources of heterogeneity are likely to be subtype of biomarker, study design, length of follow-up, sampling interval and experimental technique and equipment used to demonstrate epigenetic change. Meta-analysis may be performed if there are multiple studies reporting on individual biomarker types such as DNA methylation, histone methylation, histone acetylation, miRNA and non-coding RNA providing the same outcomes (and outcome statistic) are reported. Results will most likely be presented as different risks of progression, for example, relative risk (RR) of progression with and without the prognostic marker. Where studies have reported time to progression, HRs will be extracted where possible.

Studies of different study design and those reporting adjusted or unadjusted results will be analysed separately. RR of progression from non-dysplastic BO to BO with LGD, HGD or OADC will be calculated where possible. Adjusted results, for example, from multivariate analyses, are likely to be more informative in terms of the prognostic ability of a given marker in the context of other potential prognostic factors (such as clinical and lifestyle factors). Where meta-analyses are performed, a random-effects model will be more appropriate to account for between-study heterogeneity. Heterogeneity will also be measured statistically using the I^2 statistics and the χ^2 test. Publication bias will be assessed (by generating Funnel plots) only if more than 10 studies are present in each meta-analysis. The strength of the overall body of evidence generated by the systematic review will be assessed using the GRADE approach (Grades of Recommendation, Assessment, Development and Evaluation Working Group).[38] The full systematic review will be reported according to PRISMA guidelines.[39]

DISCUSSION

This systematic review will aim to comprehensively identify studies reporting on epigenetic changes in progressive BO. The results will help to inform future research on risk stratification and a personalised approach to endoscopic surveillance in patients with BO. The findings may inform future research into the optimisation of the Barrett's surveillance programmes using epigenetic markers as part of a multimodal screening tool.

ETHICS AND DISSEMINATION

This is a systematic review of completed studies and no ethical approval is required. Findings from the full systematic review will be submitted for publication and presentation at national and international conferences which will inform future research on risk stratification in patients with BO.

Author affiliations
[1]Department of Surgery, University of Birmingham, Birmingham, UK
[2]Birmingham Clinical Trials Unit, Institute of Applied Health Research, University of Birmingham, Birmingham, UK
[3]Institute of Applied Health Research, University of Birmingham, Birmingham, UK
[4]Department of Surgery, Heart of England Foundation Trust and Institute of Inflammation and Ageing, University of Birmingham, Birmingham, UK

Twitter Follow Andrew Beggs @adbeggs

Contributors TN, CLT, JD and OT conceived the systematic review protocol. TN, CLT, SB and JD undertook and reviewed scoping searches and contributed to the methodological development of the protocol with input from OT. TN drafted the initial manuscript and all authors (TN, CLT, JD, SB, MD, ADB and OT) were involved in its critical revision. All authors have given approval of the final version to be published. OT is the review guarantor.

Funding This work was supported by funding from the UGI Patient Support Group and QEHB Charity, QEHB, Birmingham, UK.

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement All data and information generated by this protocol will be shared by the publication and dissemination of our manuscript.

REFERENCES

1. Melhado RE, Alderson D, Tucker O. The changing face of esophageal cancer. *Cancers (Basel)* 2010;2:1379–404.
2. Chusteka Z. *Dramatic 50% rise in esophageal cancer in British men*. Medscape, 2010.
3. CRUK. *Cancer Research UK Oesophageal Cancer Survival Statistics*. 2011 (cited 1 June 2016) http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/oesophageal-cancer/survival
4. NCI. *Surveillance, epidemiology and end results programme database*. NC. Institute, Editor. 2014.
5. Fitzgerald RC, Di Pietro M, Ragunath K, *et al*. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. *Gut* 2014;63:7–42.
6. Haggitt RC, Tryzelaar J, Ellis FH, *et al*. Adenocarcinoma complicating columnar epithelium-lined (Barrett's) esophagus. *Am J Clin Pathol* 1978;70:1–5.
7. Solaymani-Dodaran M, Logan RF, West J, *et al*. *Risk of oesophageal cancer in Barrett's oesophagus and gastro-oesophageal reflux*. *Gut* 2004;53:1070–4.
8. Coupland VH, Allum W, Blazeby JM, *et al*. Incidence and survival of oesophageal and gastric cancer in England between 1998 and 2007, a population-based study. *BMC Cancer* 2012;12:11.
9. National Institute for Health and Care Excellence. *Endoscopic radiofrequency ablation for Barrett's oesophagus with low-grade dysplasia or no dysplasia—IPG496*. 2014 (cited 7 Oct 2016) https://www.nice.org.uk/guidance/ipg496/
10. Shaheen NJ, Falk G, Iyer PG, *et al*. ACG clinical guideline: diagnosis and management of Barrett's esophagus. *Am J Gastroenterol* 2016;111:30–50. quiz 51.
11. 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.
12. Rastogi A, Puli S, El-Serag HB, *et al*. Incidence of esophageal adenocarcinoma in patients with Barrett's esophagus and high-grade dysplasia: a meta-analysis. *Gastrointest Endosc* 2008;67:394–8.

13. Shaheen NJ, Sharma P, Overholt BF, *et al*. Radiofrequency ablation in Barrett's esophagus with dysplasia. *N Engl J Med* 2009;360:2277–88.
14. Overholt BF, Lightdale CJ, Wang KK, *et al*. Photodynamic therapy with porfimer sodium for ablation of high-grade dysplasia in Barrett's esophagus: international, partially blinded, randomized phase III trial. *Gastrointest Endosc* 2005;62:488–98.
15. Egger G, Liang G, Aparicio A, *et al*. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 2004;429:457–63.
16. Morey C, Avner P. Genetics and epigenetics of the X chromosome. *Ann N Y Acad Sci* 2010;1214:E18–33.
17. Besingi W, Johansson A. Smoke-related DNA methylation changes in the etiology of human disease. *Hum Mol Genet* 2014;23:2290–7.
18. Phillips T. The role of methylation in gene expression. *Nat Educ* 2008;1:116.
19. Dong X, Weng Z. The correlation between histone modifications and gene expression. *Epigenomics* 2013;5:113–16.
20. Phillips T. Small non-coding RNA and gene expression. *Nat Educ* 2008;1:115.
21. Dhir M, Montgomery EA, Glöckner SC, *et al*. Epigenetic regulation of WNT signaling pathway genes in inflammatory bowel disease (IBD) associated neoplasia. *J Gastrointest Surg* 2008;12:1745–53.
22. Garrity-Park MM, Loftus E, Sandborn WJ, *et al*. Methylation status of genes in non-neoplastic mucosa from patients with ulcerative colitis-associated colorectal cancer. *Am J Gastroenterol* 2010;105:1610–19.
23. Moriyama T, Matsumoto T, Nakamura S, *et al*. Hypermethylation of p14 (ARF) may be predictive of colitic cancer in patients with ulcerative colitis. *Dis Colon Rectum* 2007;50:1384–92.
24. Osborn NK, Zou H, Molina JR, *et al*. Aberrant methylation of the eyes absent 4 gene in ulcerative colitis-associated dysplasia. *Clin Gastroenterol Hepatol* 2006;4:212–18.
25. Kukitsu T, Takayama T, Miyanishi K, *et al*. Aberrant crypt foci as precursors of the dysplasia-carcinoma sequence in patients with ulcerative colitis. *Clin Cancer Res* 2008;14:48–54.
26. Sato F, Shibata D, Harpaz N, *et al*. Aberrant methylation of the HPP1 gene in ulcerative colitis-associated colorectal carcinoma. *Cancer Res* 2002;62:6820–2.
27. Matthews G. *Enhanced Neoplasia Detection and Cancer Prevention in Chronic Colitis (ENDCaP-C)*. NIHR, 2013.
28. Xu R, Wang F, Wu L, *et al*. A systematic review of hypermethylation of p16 gene in esophageal cancer. *Cancer Biomark* 2013;13:215–26.
29. Zhao JJ, Li H-Y, Wang D, *et al*. Abnormal MGMT promoter methylation may contribute to the risk of esophageal cancer: a meta-analysis of cohort studies. *Tumour Biol* 2014;35:10085–93.
30. Yang JZ, Ji A-F, Wang J-S, *et al*. Association between Ras association domain family 1A promoter methylation and esophageal squamous cell carcinoma: a meta-analysis. *Asian Pac J Cancer Prev* 2014;15:3921–5.
31. Wang Y, Qin X, Wu J, *et al*. Association of promoter methylation of RUNX3 gene with the development of esophageal cancer: a meta analysis. *PLoS ONE* 2014;9:e107598.
32. Fu C, Dong W, Wang Z, *et al*. The expression of miR-21 and miR-375 predict prognosis of esophageal cancer. *Biochem Biophys Res Commun* 2014;446:1197–203.
33. Fu W, Pang L, Chen Y, *et al*. The microRNAs as prognostic biomarkers for survival in esophageal cancer: a meta-analysis. *SciWorld J* 2014;2014:523979.
34. Wang Y, Wang Q, Zhang N, *et al*. Identification of microRNAs as novel biomarkers for detecting esophageal squamous cell carcinoma in Asians: a meta-analysis. *Tumour Biol* 2014;35:11595–604.
35. Findlay JM, Middleton MR, Tomlinson I. A systematic review and meta-analysis of somatic and germline DNA sequence biomarkers of esophageal cancer survival, therapy response and stage. *Ann Oncol* 2015;26:624–44.
36. Findlay JM, Middleton MR, Tomlinson I. Genetic biomarkers of Barrett's esophagus susceptibility and progression to dysplasia and cancer: a systematic review and meta-analysis. *Dig Dis Sci* 2016;61:25–38.
37. Hayden JA, Van Der Windt DA, Cartwright JL, *et al*. Assessing bias in studies of prognostic factors. *Ann Intern Med* 2013;158:280–6.
38. Atkins D, Best D, Briss PA, *et al*. Grading quality of evidence and strength of recommendations. *BMJ* 2004;328:1490.
39. Moher D, Liberati A, Tetzlaff J, Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med* 2009;151:264–9. w64.