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TOPIC HIGHLIGHT

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Biomarkers of Barrett's esophagus

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Abstract

Barrett's esophagus is the strongest risk for esophageal adenocarcinoma (EAC). Metaplasia in patients with BE may progress to dysplasia and then invasive carcinoma. Well-defined diagnostic, progressive, predictive, and prognostic biomarkers are needed to identify the presence of the disease, estimate the risk of malignant transformation, and predict the therapeutic outcome and survival of EAC patients. There are many predictive and prognostic markers that lack substantial validation, and do not allow stratification of patients with gastroesophageal reflux disease in clinical practice for outcome and effectiveness of therapy. In this short review we summarize the current knowledge regarding possible biomarkers, focusing on the pathophysiologic mechanisms to improve prognostic and therapeutic approaches.

Key words: Barrett's esophagus; Esophageal adenocarcinoma; Biomarkers

Core tip: The importance of biomarkers of Barrett's esophagus is to provide identification of the disease, estimate the risk of malignant transformation, predict the response to therapy, and indicate the overall survival-prognosis for esophageal adenocarcinoma patients. Proposed predictive and prognostic markers do not allow stratification of gastroesophageal reflux disease patients for progression, outcome, and effectiveness of therapy in clinical practice. The aim of this short review is to discuss the current knowledge regarding proposed biomarkers to improve prognostic and predictive therapeutic approaches, with a focus on the pathophysiologic mechanisms.

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INTRODUCTION

Barrett's esophagus (BE) is characterized by the replacement of squamous epithelium in the esophagus by metaplastic columnar epithelium with goblet cells[1]. BE is a well-known risk factor for esophageal adenocarcinoma (EAC), a malignancy with the most rapid increase in incidence (approximately 500%) over the past 3 decades in the Western world, and with persistently poor outcomes when diagnosed after the onset of symptoms (survival less than 20% at 5 years)[2]. An important problem in treating the patients with BE is the absence of satisfactory surveillance programs in spite of the known stages of carcinogenesis from BE to adenocarcinoma. Over the past two decades, there have been many studies attempting to identify patients with BE and predict patients with a high risk of progression to adenocarcinoma[3-6].

In this review, the definition, mechanisms of produc-

Table 1 Phases of biomarker production

Phases of biomarker validation and development
Phase 1: Biomarkers of promise are identified based on application in other cancers and elucidation of novel pathways
Phase 2: Cross-sectional studies validate the biomarker of interest to be sufficiently discriminatory and biomarker assays are standardized
Phase 3: Case-control studies with a retrospective but longitudinal design confirm the biomarker is expressed before the development of cancer
Phase 4: Prospective longitudinal studies avoid biases associated with case-control studies
Phase 5: Population-based studies show the impact of biomarker detection on disease burden and cancer control

tion, and types of biomarker in patients with BE will be summarized.

DEFINITION OF BIOMARKERS

The biomarker

A biological marker affords an indication of the condition or disease, whether normal or abnormal. It is found in the blood, body fluids, and tissues. Moreover, a biomarker may be used for assessment of the response of the body to treatment of a disease or condition[7].

Phases of biomarker identification and validation

Biomarker discovery has to pass through 5 to 6 phases before clinical application (Table 1). Phases 4, 5 and 6 present a significant challenge because of the required large sample sizes, long follow-up and high costs[8].

TYPES OF BIOMARKERS IN PATIENTS WITH BE

Genomic instability

The similarity of the genetic patterns of BE and EAC demonstrated by DNA microarray studies supported the hypothesis that BE is a step preceding EAC. The genomic instability has been shown to be a poor prognostic marker in BE patients. Chromosomal alterations, deletions, point mutations, methylation abnormalities, and loss of heterozygosity (LOH) are the main indications of genomic instability in patients with BE[9-11].

DNA abnormalities

DNA abnormalities, *e.g.*, aneuploidy or tetraploidy, assessed by flow cytometry, can be used as predictive markers in patients with BE with no or low grade dysplasia[12,13]. LOH represents the loss of normal function of one allele of a gene in which the other allele was already inactivated. In a long-term follow-up study of BE patients, a panel combining 9p LOH, 17p LOH in addition to aneuploidy and tetraploidy was a strong predictor of EAC[14].

Abnormalities of tumor loci

An important predictor of risk of dysplasia and EAC in patients with BE is LOH for p53. LOH for p53 was shown to be associated with a 16-fold increase in the risk of progression to cancer[15]. However, in another study, in patients with non-dysplastic BE, only 32.4% of patients with progression showed overexpression of p53 in their initial biopsy[16]. Furthermore, alteration of APC, a regulator of the WNT pathway, by methylation[17] and LOH[18] were found in patients with BE with a positive predictive value.

Epigenetics

Epigenetics entails post-transcriptional silencing of specific genes without a change in the DNA sequence. A variety of mechanisms are involved, including methylation and acetylation. It has been shown that hypermethylation and loss of p16, are independently associated with an increased risk of progression from intestinal metaplasia (IM) to high-grade dysplasia (HGD)[19,20].

The p16 methylation was shown to be highly prevalent in patients with BE (34%-66%)[17,19,21]. Moreover, in a multicenter study, a panel of 8 genes (*p16*, *RUNX3*, *HPP1*, *NELL1*, *TAC1*, *SST*, *AKAP12*, and *CDH13*), was used to predict the risk of progression in patients with BE. In this study, 195 patients were included and sensitivities for prediction of progression approached 50%[22].

Cell cycle predictors

A dysregulated cell cycle may lead to accumulation of genetic aberrations in most cancer cells. Cyclins are cell cycle regulator proteins, and potentially useful biomarkers for progression. In patients with BE, cyclin D1 overexpression was shown to be associated with progression to EAC[23-26]. Further research in large groups of patients is needed to confirm the predictive values of cyclins.

Proliferation abnormalities

The association between increasing proliferation and worsening of dysplasia in BE was shown in many studies[26-28], while other studies found no association[29,30]. Researchers explained the discrepancies between there results by the use different techniques, the different histological pattern between columnar and squamous epithelium, and the use of different proliferative indices. One of the important markers of cellular proliferation is Ki67. However, in a long follow-up study, Ki67-positive proliferative fractions were not associated with risk of progression[31]. Further larger studies with standardized techniques are needed to measure proliferation.

Clonal diversity in BE

Genetic instabilities may lead to multiple distinct clones. The coexistence of multiple distinct clones is called clonal diversity. In patients with BE, clonal diversity measures

Table 2 Types of biomarkers in Barrett's esophagus

	Biomarker	Method	Remarks	Ref.
Diagnostic	TFF3	IHC	To screen asymptomatic patients for BE	[49,50]
	Chromosome 7 and 17 changes	IDKA and FISH	Early stages of BE	[52]
	8q24 (*C-MYC*), 17q12 (*HER2*), and 20q13 changes	FISH	Early stages of BE	[53]
	17q11.2 (*ERBB2*)	Microarray analysis	EAC	[54]
	Serum proteomic analysis	Mass spectrometry	EAC	[55]
Predictive	P16 allelic loss	FISH	Response to therapy	[56]
	DNA ploidy abnormalities	ICDA	Covariate value for recurrence	[57]
	HSP27	IHC	No response to therapy	[58]
	Ephrin B receptor	Microarray	Response to therapy in EAC	[59]
	Genetic polymorphism	qRT-PCR	Associated with clinical outcome	[60]
	P21	IHC	Correlated with better CTX response	[61]
	P53	IHC	Correlated with better CTX response	[62]
Progression markers	ERCC1	IHC	Predicts CTX resistance	[16]
	P53	IHC	Limited efficacy as a progression marker	[13,63]
	DNA abnormalities	Flow cytometry	High risk for progression to EAC	[13]
	LOH of 157p and 9p	Flow cytometry	Predict progression to EAC	[14]
	EGFR	IHC	Overexpression in HGD and EAC	[64]
	Cyclin A	IHC	Predicts progression to dysplasia	[65]
	Cyclin D1	IHC	Risk of Progression to EAC	[19]
	Hypermethylation of p16, RUNX2,HPP1	RT-PCR	Risk of progression to EAC/HGD	[22]
	8 gene methylation panel	RT-PCR	Predicts progression to EAC/HGD	[66]
Prognostic biomarkers	Cathepsin D,AKR1D10,AKR1C2 mRNA levels	Western blot, qRt-PCR	Dysregulation predicts progression to EAC/HGD	[67]
	DCK, PAPSS2, SIRT,TRIM44	RT-PCR, IHC	4 gene signature in EAC , predict 5 year survival	[56]
	P16 loss, C-MYC gain	FISH	Associated with therapy response	[68]
	ASS expression	Microarray	Low expression associated with metastases	[69]
	MicroRNA expression profile	Microarray, RT-PCR	Low level associated with worse prognosis in EAC	[70]
	Cyclin D1	IHC, FISH	Decreased survival	[71]
	EGFR	IHC	Decreased expression associated with decreased survival	[72]
	TGF-α	IHC, ISH	High level indicates progression and metastases	[73]
	TGF-β1	RT-PCR, ELISA	High expression associated with decreased survival	[73]
	APC	PCR	High level associated with decreased survival	[74]
	COX-2	IHC	Associated with metastases and recurrence	[75]
	Telomerase	Southern-blot and PCR	Associated with decreased survival	[76]
	VEGF	IHC	Associated with metastases and decreased survival	[77]
	Cadherin	IHC	Decreased level associated with decreased survival	[78]
	TIMP	IHC, PCR	Decreased level associated with decreased survival	[79]

ACIS: Automated cellular imaging system; ASS: Argininosuccinate synthase; APC: Adenomatous polyposis coli; BE: Barrett's esophagus; COX: Cyclooxygenase; DCK: Deoxycytidine kinase; DICM: Digital image cytometry; EAC: Esophageal adenocarcinoma; EGFR: Epidermal growth factor receptor; ELISA: Enzymelinked immunosorbent assay; FISH: Fluorescence in-situ-hybridization; ICDA: Image cytometric DNA analysis; HSP27: Heat-shock protein 27; IHC: Immunohistochemistry; LOH: Loss of heterozygosity; PAPSS2: 3'-phosphoadenosine 5'-phosphosulfate synthase 2; PCR: Polymerase chain reaction; qRT: Quantitative reverse transcriptase; MLPA: Multiplex ligation dependent probe amplification; NF-κB: Nuclear factor kappa B; SIRT2: Sirtuin 2; SNP: Single nucleotide polymorphism; TFF3: Trefoil factor 3; TGF: Transforming growth factor; TIMP: Tissue inhibitors of metalloproteinases; TRIM44: Tripartite motifcontaining 44; uPA: Urokinase-type plasminogen activator; VEGF: Vascular endothelial growth factor.

were strong predictors of progression[32]. However, the complicated methodology limited the use of clonal diversity as a predictive marker.

Mitochondrial DNA

Mitochondrial DNA (mtDNA) has been implicated in the process of carcinogenesis[33]. mtDNA mutations were found in 53% of patients with BE without dysplasia[32]. In patients with BE, deletion of the mitochondrial genome (4977 bp) was found in 15.4% in IM, 40% in low-grade dysplasia, 69.2% in HGD, and 90% in paratumoral tissue[34].

FLUORESCENCE *IN-SITU* HYBRIDIZATION

Fluorescence *in situ* hybridization (FISH) is a technique which detects DNA content and loci abnormalities in the cells by fluorescent-tagged DNA probes. FISH can detect aneusomy (abnormalities of chromosome copy number), deletion, duplication, amplification and translocation at tumor suppressor loci and protooncogene loci.

In patients with BE, FISH was used to detect genetic abnormalities by investigators in different studies from multiple centers[35-39]. Detection of dysplasia in BE and identification of HGD and EAC using the FISH 4-probe set has been shown to have a reasonable sensitivity (84%-93%) and specificity (93%)[39]. In another multicenter study, polysomy detected by FISH has been shown to predict risk of progression to HGD/EAC[40].

CLASSIFICATION OF BIOMARKERS OF BE

Biomarkers of BE can be classified into 4 groups: (1)

diagnostic biomarkers; (2) biomarkers of progression; (3) predictive biomarkers; and (4) prognostic biomarkers. This classification is based on the previous intensive research, and review articles[6,41-43] (Table 2).

Diagnostic biomarkers

Diagnostic biomarkers indicate the presence of disease. The histochemical analysis of biopsies of the gastro-esophageal junction remains the conventional approach for detection and diagnosis of BE. In patients with asymptomatic BE, trefoil factor 3 combined with a non-invasive diagnostic technique has been investigated with promising results in the screening of these patients[44,45]. Further validation and assessment are needed to confirm the results of these studies.

Progression biomarkers

The degree of dysplasia in obtained biopsies is the main marker of progression of BE, although there is much intra- and inter-observer errors[46-48]. The most promising biomarkers are minichromosome maintenance 2 (MCM2) expression pattern and LOH on distinct gene loci, especially at 17p. The cost and intensive laboratory time limit the use of these markers in clinical practice.

Predictive biomarkers

These biomarkers predict the response to therapy. A limited number of predictive biomarkers are available (Table 2) and this category is in need of further intensified research.

Prognostic biomarkers

These biomarkers indicate overall survival and prognosis of EAC. The majority of biomarkers are in this category. Prognostic biomarkers include growth signals, insensitivity to growth inhibitory signals, markers of evasion of programmed cell death, limitless replicative potential (telomerase), markers of sustained angiogenesis, markers of invasion and metastasis, marker of tumor differentiation, and cancer-related inflammation (Table 2).

Biomarkers in the clinical field: problems and obstacles

Much work is needed to set up clinical trials of biomarkers as this requires cooperation between clinical researchers and experts in molecular techniques. Moreover, the validation of a biomarker passes through 5 phases and requires multicenter studies, with prohibitive costs and long-term follow-up.

The method of specimen collection is another challenge. While microarray studies require special equipment and may not be easy to access by clinical scientists, molecular profiling using formalin-fixed paraffin-embedded specimens is interesting to researchers because of easy availability of specimens. In patients with hepatocellular carcinoma, the use of large scale (> 6000) gene profiling resulted in high quality data even from specimens archived for as long as 24 years[49].

The lack of prospective controlled trials is another important problem attributed to high costs and the need for large sample sizes. Moreover, the lack of reproducibility of assays between laboratories represent another obstacle for identification of clinically useful cancer biomarkers[50]. The reanalysis of DNA microarray studies showed that the selection of patients had an impact on the predictor role of genes in prognosis[51]. Careful interpretation of biomarker studies is needed by using large datasets such as DNA microarray repositories.

CONCLUSION

A biomarker for BE should help in population screening, improve the surveillance of patients with BE, and identify the prognostic groups and best therapy once EAC develops. Many biomarkers have been intensively studied and accurately predict the progress of BE to EAC. The MCM2 expression pattern, LOH on distinct gene loci, especially at 17p, hypermethylation of p16 and the expression pattern of P53 are promising markers especially for progression of the disease. Important prognostic biomarkers include cyclin D1, Ki-67, transforming growth factor-α, adenomatous polyposis coli, cyclooxygenase-2, telomerase and vascular endothelial growth factor. Till now, no biomarker has been able to replace the current gold standard of dysplasia in routine clinical practice. Panels of biomarkers seem to be better in predicting progression more accurately. The issue of costs and practicality of biomarkers should be considered before research is performed. A model incorporating clinical data and biomarkers will be promising and can accurately predict the risk of progression, prognosis or response to therapy. Similar models have been used in other cancers and diseases such as the Nottingham prognostic index for breast cancer and MELD score for liver disease. After generation and validation of such a model, it should then be rigorously validated in a large cohort of patients in a prospective fashion.

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