



Yeng S Ang, Dr., MD, Series Editor

Biomarkers in Barrett's esophagus and esophageal adenocarcinoma: Predictors of progression and prognosis

Chin-Ann J Ong, Pierre Lao-Sirieix, Rebecca C Fitzgerald

Chin-Ann J Ong, Pierre Lao-Sirieix, Rebecca C Fitzgerald, MRC Cancer Cell Unit, Hutchison-MRC Research Centre, Cambridge, CB20XZ, United Kingdom

Author contributions: Ong CAJ, Lao-Sirieix P and Fitzgerald RC performed the literature review, critically analyzed the evidence and wrote the paper.

Correspondence to: Rebecca C Fitzgerald, MD, MRC Cancer Cell Unit, Hutchison-MRC Research Centre, Box 197/ Hills Road, Cambridge, CB20XZ, United Kingdom. rcf@hutchison-mrc.cam.ac.uk

Telephone: +44-1223-763287 Fax: +44-1223-763296

Received: May 27, 2010 Revised: July 28, 2010

Accepted: August 4, 2010

Published online: December 7, 2010

Key words: Barrett's esophagus; Esophageal adenocarcinoma; Esophageal dysplasia; Prognosis

Peer reviewers: Dr. Katerina Dvorak, Research Assistant Professor, Cell Biology and Anatomy, The University of Arizona, 1501 N. Campbell Ave, Tucson, AZ 85724, United States; Zhiheng Pei, MD, PhD, Assistant Professor, Department of Pathology and Medicine, New York University School of Medicine, Department of Veterans Affairs, New York Harbor Healthcare System, 6001W, 423 East 23rd street, New York, NY 10010, United States; Leonidas G Koniaris, Professor, Alan Livingstone Chair in Surgical Oncology, 3550 Sylvester Comprehensive Cancer Center (310T), 1475 NW 12th Ave., Miami, FL 33136, United States

Ong CAJ, Lao-Sirieix P, Fitzgerald RC. Biomarkers in Barrett's esophagus and esophageal adenocarcinoma: Predictors of progression and prognosis. *World J Gastroenterol* 2010; 16(45): 5669-5681 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v16/i45/5669.htm> DOI: <http://dx.doi.org/10.3748/wjg.v16.i45.5669>

Abstract

Barrett's esophagus is a well-known premalignant lesion of the lower esophagus that is characterized by intestinal metaplasia of the squamous epithelium. It is clinically important due to the increased risk (0.5% per annum) of progression to esophageal adenocarcinoma (EA), which has a poor outcome unless diagnosed early. The current clinical management of Barrett's esophagus is hampered by the lack of accurate predictors of progression. In addition, when patients develop EA, the current staging modalities are limited in stratifying patients into different prognostic groups in order to guide the optimal therapy for an individual patient. Biomarkers have the potential to improve radically the clinical management of patients with Barrett's esophagus and EA but have not yet entered mainstream clinical practice. This is in contrast to other cancers like breast and prostate for which biomarkers are utilized routinely to inform clinical decisions. This review aims to highlight the most promising predictive and prognostic biomarkers in Barrett's esophagus and EA and to discuss what is required to move the field forward towards clinical application.

INTRODUCTION

Barrett's esophagus is defined as an esophagus in which the distal portion of the normal squamous lining has been replaced by a metaplastic columnar epithelium. In order to make a diagnosis of Barrett's esophagus, a segment of columnar metaplasia of any length must be visible endoscopically above the esophagogastric junction and be confirmed or corroborated histologically^[1]. This condition usually develops in the context of longstanding, severe gastroesophageal reflux disease (GERD)^[2], and is the only recognized precursor lesion for development of esophageal adenocarcinoma (EA). The incidence of EA arising from Barrett's esophagus is variable, depending on the grade of dysplasia associated with it. The risk of progression to cancer increases gradually from 0.5% per year for non-dysplastic Barrett's, to 13% in low-grade dysplasia (LGD) and 40% in high-grade dysplasia (HGD)^[3,4].

In Barrett's esophagus, it is widely accepted that there are three main histological subtypes. They include epithelium that comprises mainly a gastric fundus subtype with parietal and chief cells; a junctional (cardia) subtype with mucus-secreting glands; and the distinctive metaplastic columnar epithelium with intestinal-type goblet cells^[1,5]. These three histological subtypes occupy different zones in the esophagus. The intestinal-type metaplasia with goblet cells is found most proximally next to the squamous epithelium, followed by the junctional (cardia) subtype in the middle, and the gastric fundus subtype most distally. The relevance of this subgrouping of the histological subtypes of Barrett's esophagus lies in the potential to develop malignancy. The fundic subtype has a very low risk of developing EA malignant potential, whereas the metaplastic columnar epithelium with intestinal-type goblet cells and the junctional (cardia) type have a more significant risk of malignant transformation^[6,7]. This concept is important as this together with the problem of defining Barrett's esophagus based on location and length of metaplastic epithelium has led to a detailed discussion in the American Gastroenterological Association Institute technical review on Barrett's esophagus. This meeting redefined Barrett's esophagus as "the condition in which any extent of metaplastic columnar epithelium that predisposes to cancer development replaces the stratified squamous epithelium that normally lines the distal esophagus"^[8]. However, it is slowly becoming apparent that the risk for development of EA is not solely limited to the intestinal type and that better designed and powered studies are required to assess properly the true risk of progression in each subtype^[9].

During the development of EA, the epithelium accumulates multiple molecular abnormalities and becomes increasingly dysplastic^[10]. The diagnosis of dysplasia allows the progression from Barrett's esophagus to EA to be monitored by endoscopic surveillance biopsies with the aim of intervening prior to the development of invasive adenocarcinoma. Although randomized controlled evidence is lacking, EA detected *via* this strategy appears to confer a much better prognosis, as surveillance detected disease is often at an early stage prior to lymph node involvement^[11,12].

There are a number of problems with this current clinical algorithm. First of all, a significant proportion of patients with Barrett's esophagus are undiagnosed^[13-16], and therefore, will not benefit from any cancer prediction strategies. Second, surveillance is not proven to reduce population mortality and is based on the subjective assessment of dysplasia, which has inter and intra-observer error^[17-19]. Lastly, because most patients with Barrett's esophagus are at extremely low risk of developing EA^[20], the majority are having unnecessary surveillance, which is cumbersome both for the clinician and the patient, and poses a strain on the healthcare system. A recent review to assess the cost-effectiveness of surveillance of Barrett's esophagus based on a Markov model has revealed that surveillance of Barrett's esophagus for all grades of dysplasia does more harm than good when compared to

no surveillance^[21]. This report has suggested that surveillance does not produce more quality-adjusted life years than no surveillance, and that there is no apparent survival advantage of cancer detected by surveillance due to a high recurrence rate and increased mortality from surgical interventions. It is hoped that biomarkers assayed in readily obtainable biological samples, such as blood or endoscopic biopsies, can be identified to improve the clinical management at each stage in the disease. Screening biomarkers could enable unidentified cases of Barrett's esophagus to be diagnosed in the population (Figure 1, green arrow), whereas predictive biomarkers could be used as adjuncts or to replace the current surveillance program for the detection of dysplasia, as well as potentially being able to predict which patients are at high risk of developing cancer in the future (Figure 1, blue arrow). For patients presenting *de novo* with EA, prognostic biomarkers could be useful to determine the best therapeutic approach and prognosis (Figure 1, red arrow). In addition, biomarkers might have a role in determining response to treatment including chemopreventive agents, endoscopic treatments for patients with Barrett's, and the use of molecular targeted therapy for those with cancer.

CLINICAL BIOMARKERS

Clinical biomarkers can be defined as a characteristic that can be objectively measured or evaluated as an indicator of normal biological processes, pathological processes or a response to a therapeutic intervention^[22]. Importantly, the quantification of biomarkers should aid, improve or alter clinical management. The criteria required for adoption of biomarkers into clinical use are not well defined. Therefore, the Early Detection Research Network (EDRN) has defined five stages for development of biomarkers for risk of progression^[23] and similarly, McShane *et al*^[24] have recently published recommendations for prognostic tumor marker development (Figure 2). Despite the recommendation of different robust algorithms for biomarker development, fewer than 12 biomarkers have been approved by the US Food and Drug Administration for monitoring response, surveillance and recurrence of cancer at the current time^[25]. This is alarming as thousands of biomarkers have been declared to be useful for diagnosis, surveillance or therapeutic markers for diseases. Most of these biomarkers do not progress to clinical practice either due to problems developing accurate assays or because the biomarker lacks sufficient sensitivity and specificity in validation studies^[26]. Clearly, a large concerted effort is needed to advance the field of biomarker discovery and clinical implementation.

Biomarkers in Barrett's esophagus and EA are mostly selected due to their role in carcinogenesis. It is clear that during the transition from metaplasia to carcinoma, many molecular alterations take place and they operate together to influence the pathogenesis of dysplasia and EA. Biomarkers can be identified and investigated for their clinical applicability using two different complementary

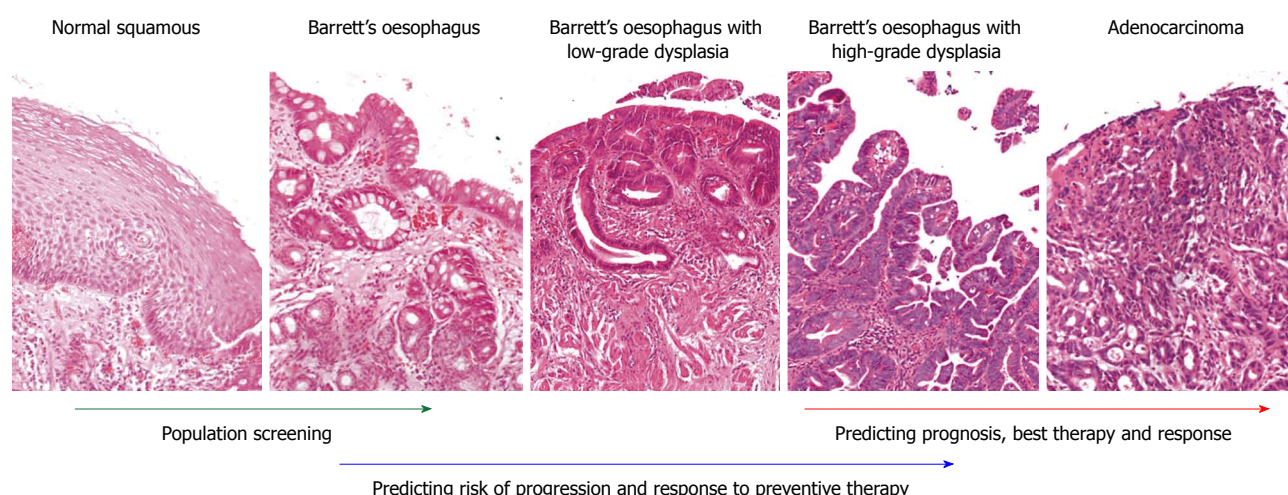


Figure 1 Transition of squamous epithelium to intestinal metaplasia, dysplasia and adenocarcinoma, with potential useful biomarkers at each stage of the disease. The left-most panel shows normal stratified squamous epithelium. The second panel shows Barrett's esophagus without dysplasia, with the presence of goblet cells. The third and fourth panels show Barrett's esophagus with low-grade dysplasia and high-grade dysplasia, whereas the last panel shows adenocarcinoma.

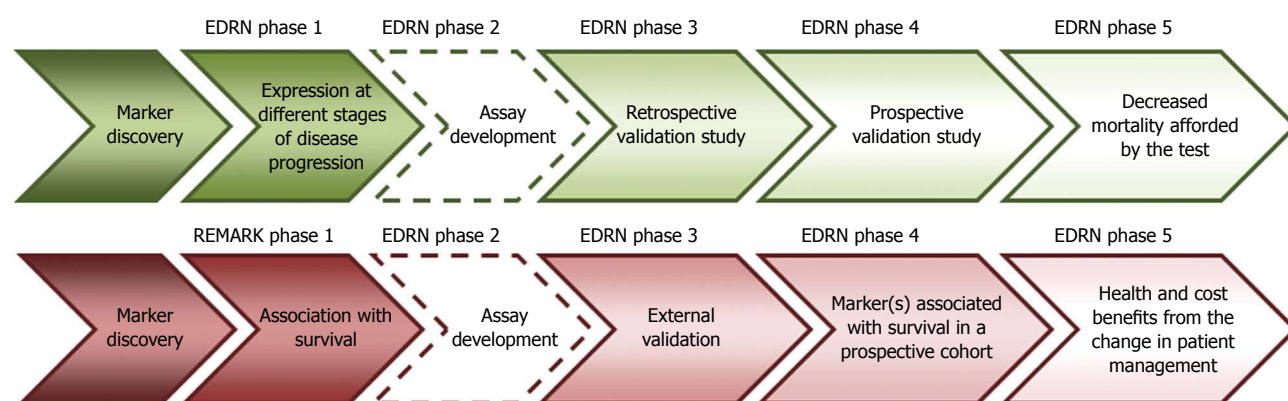


Figure 2 Phases of diagnostic and prognostic biomarker development proposed by the Early Detection Research Network and reporting recommendations for tumor marker prognostic studies before clinical implementation^[23,24]. EDRN: Early Detection Research Network; REMARK: Reporting recommendations for tumor marker prognostic studies.

approaches^[27]. The first approach is to identify candidate biomarkers from what is currently understood about the disease process. This is a comparatively inexpensive way to identify putative biomarkers and possibly allow for faster clinical implementation of the biomarker. The second method is to use a global screening approach without an *a priori* hypothesis. This has become possible due to the rapid expansion of “omics” technologies, including gene expression analysis, epigenetics, proteomics and single nucleotide polymorphism (SNP)-based platforms. The availability of microarray databases and other datasets on the internet also allows for the interrogation of multiple datasets to identify potential biomarkers. For example, Lao-Sirieix *et al.*^[28] have identified trefoil factor 3 (TFF3) as a promising biomarker to screen asymptomatic patients for Barrett's esophagus by comparing three publically available microarray databases. However, this approach requires an intensive validation process due to the potential for false discovery and can potentially be expensive and not reproducible between laboratories.

This review focuses on two main areas: (1) biomark-

ers predictive of progression in Barrett's esophagus, which it is hoped could transform the current surveillance program; and (2) prognostic biomarkers in EA.

PROMISING BIOMARKERS IN SURVEILLANCE OF BARRETT'S PATIENTS

Many biomarkers aimed at predicting progression in Barrett's patients have emerged over several years of research because it is appreciated that current clinical and endoscopic criteria are unable to predict which patients are likely to progress to EA. Biomarkers in Barrett's esophagus can be used for population screening and early detection of disease, confirmation of diagnosis of disease and prediction of risk of progression, which determine the prognosis of patients once adenocarcinoma develops and predict the effectiveness of therapy. Table 1 shows a summary of the biomarkers that have been most extensively investigated and their potential as clinical biomarkers. In studies evaluating the efficacy of the proposed biomarkers to determine the risk of progression from Barrett's

Table 1 Summary of the most promising biomarkers for identifying patients with Barrett's esophagus at high risk of developing esophageal adenocarcinoma

Surveillance biomarker	Highest EDRN stage	Study size (n) ¹	Findings	Statistical significance	Ref.
HGD	4	15	Progression to EA in 4 out of 15 patients with unifocal HGD	RR not available	[29]
		485	20 patients with HGD treated with omeprazole only developed EA	RR not available	[30]
		327	33 out of 76 patients with HGD developed EA	RR 28 (95% CI: 13-63)	[31]
		1099	12 out of 75 patients with HGD developed EA	RR 12.1 (95% CI: 5-29.4)	[32]
Aneuploidy and LOH (Reid Panel)	4	243	Panel of biomarkers (LOH of 17p and 9p and DNA abnormalities) can best predict progression to EA	RR 38.7 (95% CI: 10.8-138.5)	
			LOH of 17p alone	RR 10.6 (95% CI: 5.2-21.3)	[33]
			LOH of 9p alone	RR 2.6 (95% CI: 1.1- 6.0)	
			Aneuploidy alone	RR 8.5 (95% CI: 4.3-17.0)	
p53 positivity by immunohistochemistry	3		Tetraploidy alone	RR 8.8 (95% CI: 4.3-17.7)	
		164	Diffuse or intense TP53 staining elevated in patients who developed EA compared to controls	OR 11.7 (95% CI: 1.93-71.4)	[34]
		48	3 out of 5 patients with low grade dysplasia who progressed to high grade dysplasia had positive p53	RR not available	[35]
Mcm2	3	27	Ectopic luminal surface expression predictive of progression to HGD or EA	OR 136 (95% CI: 7.5-2464)	[36]
Cyclin A	3	48	Ectopic luminal surface expression predictive of progression to HGD or EA	OR 7.6 (95% CI: 1.6-37)	[37]
Methylation markers	3	53	Hypermethylation of <i>p16</i> (cyclin-dependent kinase inhibitor 2A), <i>RUNX3</i> (Runt-related transcription factor 3) and <i>HPP1</i> (transmembrane protein with EGF-like and two follistatin-like domain 2) associated with an increased risk of progression to high grade dysplasia or EA	OR 1.74 (95% CI: 1.33-2.2), 1.80 (95% CI: 1.08-2.81) and 1.77 (95% CI: 1.06-2.81), respectively	[38]
		195	A 8 gene methylation panel in combination with age could predict half of progressors to HGD or EA who would not have been diagnosed without the use of the panel	RR not available	[39]

¹Study size includes all patients in study and findings are extracted when relevant. Mcm2: Minichromosome maintenance protein 2; EA: Esophageal adenocarcinoma; HGD: High-grade dysplasia; LOH: Loss of heterozygosity; EDRN: Early Detection Research Network; RR: Relative risk; OR: Odds ratio; CI: Confidence interval.

esophagus to dysplasia and cancer, the odds ratio and relative risks are included whenever data were available in order to give a representation of the usefulness of the biomarkers.

DYSPLASIA

Dysplasia has been assessed as part of routine clinical practice for > 20 years. Although the assessment of dysplasia cannot be measured objectively, it is still considered a biomarker by most institutions, and is the current gold standard for determining the risk for cancer progression. The current dysplasia grading system is the Vienna classification, which divides patients into no dysplasia, LGD and HGD^[40]. Due to its routine use, very few studies have been performed to document formally its predictive power. A recent meta-analysis has shown that the incidence of EA in patients undergoing surveillance for Barrett's esophagus rises in a stepwise manner using dysplasia as a biomarker. The incidence of EA was reported to be 5.98 per 1000 patient years, 16.98 per 1000 patient years and 65.8 per 1000 patient years in Barrett's patients without dysplasia, and with LGD and HGD, respectively^[4]. However, histological differentiation of the different grades of dysplasia in Barrett's patients presents one of the most difficult tasks for the pathologist. In one study, 50% of Barrett's patients who were identified to have LGD by general pa-

thologists were misdiagnosed. Forty-two percent of these misdiagnosed cases had only Barrett's esophagus without dysplasia, and 8% had HGD^[41]. It is clear that histological differentiation between non-dysplastic Barrett's esophagus and LGD in particular is fraught with difficulties with poor intra- and inter-observer agreement.

HGD is known to be a surrogate marker for the high likelihood of progression to EA. Following diagnosis of HGD, endoscopic or surgical intervention is usually considered. Therefore, confirmation by two independent pathologists is a pre-requisite. As a result of the practice for intervention once HGD is detected, data on progression to EA have become much harder to obtain. Studies have shown that the risk of progression to EA ranges from 16% to 59%^[31,32] and a proportion of patients in whom HGD is detected will already harbor invasive adenocarcinoma^[29,32], although with intensive biopsy protocols and high definition endoscopes, this should no longer be so likely. A more ideal biomarker would be one that is less subjective and that appears earlier in the pathogenetic process, so that intervention could be considered for the highest risk patients earlier in the course of their disease. The evaluation of dysplasia is now well established and it has been suggested that other promising biomarkers are more likely to be used in conjunction with the current system than to replace the histopathological assessment of dysplasia^[42].

DNA CONTENT ABNORMALITIES AND LOSS OF HETEROZYGOSITY

The use of DNA content abnormalities (aneuploidy and tetraploidy) and loss of heterozygosity (LOH) as biomarkers to predict progression of Barrett's esophagus to EA has been intensively studied by the Reid group. DNA content abnormalities are a well-known phenomenon in cancer biology. A normal cell contains 46 chromosomes, commonly referred to as 2N, and aneuploidy refers to the state in which cells have an abnormal number of chromosomes. Tetraploidy, on the other hand, specifically refers to cells that have double the number of chromosomes compared to normal cells (4N). In Barrett's esophagus, numerous studies have correlated aneuploidy and specific DNA abnormalities with the progression of Barrett's esophagus to EA^[31,43-45], with Reid *et al.*^[31] producing the best results by combining DNA content abnormalities with LOH. Galipeau *et al.*^[44] have demonstrated that increased 4N (G2/tetraploid) cell populations predict progression to aneuploidy, and that the development of 4N abnormalities is interdependent with inactivation of the *p53* gene. Using flow cytometry and histology in a systematic endoscopic biopsy protocol, Reid *et al.*^[31] first described the use of aneuploidy and increased 4N fractions as biomarkers to identify subsets of patients with Barrett's esophagus at low and high risk of developing EA. Using a cut-off for 4N fractions of > 6% as abnormal, Reid has reported that the relative risk of cancer for these patients compared to those below this cut-off value was 7.5 (95% CI: 4-14). In addition, patients who had baseline aneuploidy had a relative risk of cancer of 5 (95% CI: 2.7-9.4) compared to patients who did not have baseline aneuploidy.

p16 and *p53* are two commonly studied tumor suppressor genes that reside on chromosome 9p and 17p, respectively. These two tumor suppressor genes can be silenced *via* LOH, mutations and DNA methylation. Silencing of the *p16* allele is thought to be one of the earliest events in Barrett's esophagus, which results in clonal expansion^[46]. However, a recent study by Leedham *et al.*^[47] has demonstrated that Barrett's esophagus can arise from multiple independent clones, which results in clonal heterogeneity. This study was performed by investigating individual crypts microdissected from esophagectomy specimens that contained adenocarcinoma and associated dysplasia, to detect clonal heterogeneity not detected by whole biopsy analysis. Overall, *p16* by itself is unlikely to be an ideal biomarker to predict progression because it appears too early in the pathogenesis, and it has been shown that there is no evidence of association between silencing of *p16* and grade of dysplasia^[46]. *p53* LOH, on the other hand, provides one of the most promising biomarkers to predict progression of Barrett's esophagus, as part of the Reid panel. *p53* is a nuclear tumor suppressor protein that is responsible for the integrity of the genetic sequence. Any damage to DNA should result in increased expression of *p53*, which causes cells to arrest at the G1 phase to allow for DNA repair, and if this is not possible, then apoptosis ensues. Silencing of *p53* can occur *via* LOH or

mutation of the genetic sequence, thus removing the self repair mechanism. Reid *et al.*^[48] have performed a prospective cohort study in 325 patients with Barrett's esophagus, and have demonstrated that LOH of chromosome 17p(*p53*) significantly increased the risk of progression to cancer (relative risk of 16, 95% CI: 6.2-39). In addition, Galipeau *et al.*^[33] have demonstrated that LOH of 17p can be combined with LOH at 9p, DNA content abnormalities and aneuploidy to form a panel of biomarkers to predict better progression of Barrett's esophagus. This panel of biomarkers provides the best predictor of progression to EA to date (relative risk of 38.7, 95% CI: 10.8-138.5). Each individual marker in the panel could in itself predict progression to EA with varying RR (Table 1), but when combined together in the Reid panel, they can most accurately predict progression to EA.

The panel of biomarkers that incorporate DNA abnormalities and LOH, which have been developed by the Reid group, are not easy to apply to the clinical setting. Efforts have therefore been made to develop alternatives. Fang *et al.*^[45] and Vogt *et al.*^[49] have tried to circumvent the problem of a high level technical expertise being required and the laboratory variability associated with flow cytometry, by using image cytometric DNA analysis in smaller studies. In these studies, they have concluded that image cytometry can provide a more sensitive marker than using HGD to identify groups of patients who are likely to progress to EA, and have highlighted that image cytometry has significant advantage over flow cytometry in terms of costs and practicality. These findings, while promising, still require validation with a much larger sample size. The development of high-fidelity DNA histograms generated by automated software to measure aneuploidy further strengthens the role of DNA abnormalities as a biomarker to predict progression in Barrett's patients^[50,51]. Other interesting novel techniques to measure aneuploidy and other chromosomal aberrations have also been described in the literature. Li *et al.*^[52] have demonstrated that the number of SNPs was highly correlated with chromosomal abnormalities in Barrett's esophagus and EA, and have suggested that SNP-based genotyping could possibly be used to stratify the cancer risk in patients with Barrett's esophagus.

As mentioned previously, the use LOH as biomarkers is not without its own problems. The detection of LOH is complex and requires the collection of snap frozen samples, followed by extraction of DNA and an amplification step prior to polymerase chain reaction analysis^[53]. This is in addition to the high costs needed to build and maintain facilities to enable the use of this panel of biomarkers in routine medical institutions. An alternative method would be to use fluorescence *in situ* hybridization (FISH) to detect LOH, but this method is limited by poor sensitivity (68.4%) when compared to genotyping^[54].

Immunostaining for *p53* provides another alternative to genotyping of chromosome 17p to predict progression of Barrett's esophagus because the presence of *p53* mutations can often cause protein accumulation, which allows for detection by immunohistochemistry^[34,35]. Although

the use of immunostaining of *p53* allows easy clinical implementation, its efficacy as a biomarker is limited, and positive staining was only seen in one third of patients in a nested case-control study to evaluate the efficacy of immunostaining for *p53* as a marker to predict progression^[34]. This is because staining for *p53* does not always correlate with mutations. In instances in which mutations result in deletion or truncation of *p53*, it will not be detected by immunostaining.

In summary, the detection of aneuploidy and DNA content abnormalities in the Reid panel appears to be one of the most promising biomarker panels to detect the progression of Barrett's esophagus to EA. However, technical difficulties that have hindered the use of analysis of DNA content abnormalities in the Reid panel need to be addressed. SNP analysis or image cytometry are other alternative techniques used to measure aneuploidy and other chromosomal aberrations but remains to be validated in larger studies.

PROLIFERATION MARKERS

Dysplasia is typically described as being associated with abnormal cellular proliferation and differentiation^[55,56]. Our laboratory and others have demonstrated abnormal surface staining of markers of proliferation [minichromosome maintenance protein (Mcm) 2, 5 and Ki67] in dysplastic Barrett's mucosa^[36,55,56]. This finding has served as the basis for the use of aberrant surface expression of Mcm2, together with a brushing technique to predict progression in patients with Barrett's esophagus^[36]. However, large prospective studies are needed before they can be used in routine clinical practice.

CELL CYCLE MARKERS

Members of the cyclin family such as cyclin A and D are also interesting biomarkers for Barrett's esophagus. Cyclin D is a proto-oncogene protein and overexpression in Barrett's esophagus results in inappropriate phosphorylation and inactivation of p105-Rb. Increased expression of cyclin D has been implicated in the predisposition to transform from metaplastic epithelium to cancer, and can possibly be a useful biomarker in identifying patients with Barrett's esophagus at high risk of developing EA^[57,58]. Bani-Hani *et al.*^[58] have performed a case-control study and have shown that Barrett's patients who are positive for cyclin D detected *via* immunohistochemistry were more likely to develop EA (OR: 6.85, 95% CI: 1.57-29.91). These findings were however not replicated in a larger population-based case-control study performed by Murray *et al.*^[34]. In that study, only immunohistochemical detection of *p53* has been shown to be a useful biomarker for malignant progression in Barrett's esophagus. Cyclin A is expressed just before the beginning of DNA synthesis and is an important check mechanism in the G1-S transition of the cell cycle. In a case-control study, surface expression of cyclin A in Barrett's esophagus samples has been shown to be correlated with the degree of dysplasia, and

patients with biopsies that express cyclin A at the surface were more likely to progress to EA than those who did not (OR: 7.5, 95% CI: 1.8-30.7)^[37]. Prospective studies are required to determine properly the usefulness of cyclins as predictive biomarkers.

EPIGENETIC CHANGES

Epigenetic changes (or non-DNA sequence changes) in the form of hypomethylation, hypermethylation and alteration to histone complexes have also been found to be implicated in the pathogenesis of Barrett's esophagus and EA^[38,59]. Hypermethylation of promoter CpG island is thought to be the cause of transcriptional silencing of tumor suppressor genes such as *CDKN2A* (*p16*), *APC*, *CDH1* (E-cadherin), and *ESR1* (ER, estrogen receptor α)^[59]. Hypermethylation of these genes is usually found in a large contiguous field, which suggests possible clonal expansion of hypermethylated cells or hypermethylation of a field of metaplastic cells^[59]. Further work on the methylation status of promoter regions of genes has revealed that methylation of *p16* (OR: 1.74, 95% CI: 1.33-2.20), *RUNX3* (OR: 1.80, 95% CI: 1.08-2.81) and *HPP1* (OR: 1.77, 95% CI: 1.06-2.81) in patients with non-dysplastic Barrett's esophagus and LGD were independent risk factors for progression to HGD and EA^[38]. More recently, Jin *et al.*^[60] have demonstrated that a methylation biomarker panel that comprises eight genes could accurately determine the risk of progression in patients with Barrett's esophagus in a retrospective, multicenter validation study. In that study, promoter methylation levels of eight genes were quantified by methylation-specific PCR in patients who did not progress ($n = 145$) compared to those who did progress ($n = 50$) to HGD or EA. Receiver operating characteristics curves were constructed to evaluate the usefulness of the eight-gene methylation panel and the authors have concluded that, with specificity set at 0.9, the eight-gene methylation panel in combination with age predicted half the progressors who would not have been diagnosed without using these biomarkers. Similarly, a recent study by Wang *et al.*^[61] has shown that hypermethylation of *p16* and *APC* was a good predictor of progression to HGD or EA [OR: 14.97, 95% CI (1.73, inf)]. The fact that methylation changes in DNA occur early in the progression from Barrett's esophagus to dysplasia suggest that they could potentially be used as biomarkers to predict which groups of patients are likely to progress to dysplasia and EA^[59,62]. However, the main problem of the utility of hypermethylation as biomarkers lies in the fact that techniques that have been applied for detection of epigenetic changes require enzyme digestion, affinity enrichment or bisulfite treatment before probe hybridization or sequencing can be done to detect methylation in samples. These arrays of techniques are far too technically demanding and time consuming for routine utilization in the clinic^[63-72].

PROGNOSTIC BIOMARKERS IN EA

The overall 5-year survival for EA remains < 14%^[73].

The current staging of EA is the internationally recognized TNM system^[74], which is based exclusively on the anatomical extent of the disease. This is assessed using a combination of tumor depth (T), number of lymph nodes involved (N), and presence or absence of metastasis (M). The TNM system remains useful for staging of esophageal tumors because patients with more advanced stage disease clearly do worse than those in the early stage of the disease. For patients deemed to have potentially curative disease (T3N1 or less), surgical treatment with or without chemotherapy provides the only chance of cure, but it is highly invasive and has a high morbidity rate. Biomarkers that can accurately predict the prognosis of patients with this disease could aid in the selection of patients most likely to benefit from surgery. In addition, it is also hoped that biomarkers can identify different subgroups of tumors that will benefit from specific treatment, including molecularly targeted treatments.

Prognostic biomarkers in patient with EA have commonly been studied to determine the association with the following outcome and tumor characteristics: (1) survival; (2) lymphovascular invasion and metastasis; and (3) response to chemotherapy and radiotherapy.

Traditional candidate approaches for analyzing gene and protein expression in cancer have identified a large number of biomarkers that have important prognostic value. These biomarkers can be considered in terms of the six classical hallmarks described by Hanahan *et al.*^[75], with inflammation added as the seventh hallmark recently. They include: (1) self sufficiency in growth signals; (2) insensitivity to growth inhibitory (antigrowth) signals; (3) evasion of programmed cell death (apoptosis); (4) limitless replicative potential; (5) sustained angiogenesis; (6) invasion and metastasis; and (7) cancer-related inflammation.

Table 2 gives an overview of the biomarkers in each category and their association with survival or surrogate measures of prognosis. This list is not exhaustive but it highlights the important biomarkers that have been investigated and reported to be prognostic. A recent review by Lagarde *et al.*^[76] has described in greater detail many of these biomarkers and their molecular basis. It is well known that many of these molecular alterations occur in tandem during the progression of Barrett's esophagus to EA and are present to varying degrees. These biomarkers have been shown to be associated with survival or tumor characteristics, but subsequent replication of findings, as required for the EDRN validation of biomarkers, is often lacking. It is highly unlikely that any of these markers by itself can predict survival accurately because several molecular alterations can operate together to influence the pathogenesis of EA. Again, generating panels of biomarkers to create a molecular signature in EA could be useful in determining the prognosis of patients with EA.

MOLECULAR SIGNATURE OF EA

Several studies have used microarray technologies to generate molecular signatures that correlate with overall

survival, lymph node involvement or response to chemotherapy. The advantage of using these methods is that they allow the hypothesis-free interrogation of many targets simultaneously. Table 3 gives a summary of the molecular signatures discovered by microarray technology, including the methodology used. However, despite the number of studies, none of these molecular signatures or techniques to stratify patients with EA has yet reached clinical utility. This is in contrast to other cancers for which prognostic signatures are starting to be used in the clinical setting^[101-103]. In EA, molecular signatures have usually been generated from underpowered cohorts and many studies have combined molecular profiling of both EA and squamous cell carcinoma of the esophagus in the same study. It is known that the molecular profile of squamous cell carcinoma and EA is different^[104,105], and for accurate prognosis, studies should differentiate between these two types of tumors. An additional problem, which is not dissimilar to biomarkers discovered for the transition of Barrett's esophagus to EA, is that the technique used might not be applicable to routine laboratories and will therefore be expensive. It is therefore important that researchers also consider how best to apply molecular biomarkers to the clinic, and they should consider validation using methods such as immunohistochemistry. Whichever method is used, data reproducibility and validation in independent samples are perhaps the most important factors to determine whether molecular signatures are adopted for clinical application. This problem is becoming increasingly recognized, and many reviews have reiterated the need for validation of molecular signatures and the development of assays that have general clinical applicability^[106-112].

CONCLUSION

The pathogenesis from Barrett's esophagus to EA is highly complex. Multiple molecular alterations occur during this process, which leads to a heterogeneous tumor by the time that EA develops. Biomarkers can complement the current clinical management of Barrett's esophagus and its transition to EA in three main ways. They can be used to: identify patients not previously diagnosed with Barrett's esophagus *via* population screening; improve the surveillance of patients with Barrett's esophagus; and identify prognostic groups and best therapy once EA develops.

There has already been a tremendous amount of research done to create an ideal biomarker or panel of biomarkers to predict accurately progression of Barrett's esophagus to dysplasia or EA. This is in conjunction with large amounts of resources and money spent in laboratories and in clinical trials as the research is being conducted. Although no biomarkers have been able to replace the current gold standard of dysplasia as a biomarker in routine clinical practice, it is reassuring to know that certain biomarkers hold great promise to transit from the bench to the bedside. It is becoming increasingly clear that one biomarker by itself is highly unlikely to predict progression with high sensitivity and

Table 2 Summary of the biomarkers and the prognostic impact in esophageal adenocarcinoma

Category of cell alteration	Biomarker	Sample size (n)	Endpoint	Findings	Statistical significance	Ref.
Self sufficiency in growth signals	Cyclin D EGFR	124	Survival	2 of 3 genotypes confers a poorer overall survival	$P = 0.0003$	[77]
		103	Survival	Expression showed a trend towards a correlation with poorer overall survival	$P = 0.07$	[78]
		75	Survival	Decreased expression correlated with poorer survival on univariate analysis only	$P = 0.034$	[79]
	Ki-67	59	Survival	Low levels (< 10%) of staining correlated with poorer survival	HR: 3.9, $P = 0.02$	[80]
	Her2/neu	63	Survival	Amplification detected by FISH correlated with poorer survival	$P = 0.03$	[81,82]
	TGF- α	61	Survival	Low levels significantly correlated with cancer specific death	$P = 0.03$	[83]
		87	Tumor progression, lymph node metastasis	High levels significantly correlated with: Tumor progression Lymph node metastasis	$P = 0.025$ $P < 0.05$	[84]
Insensitivity to growth inhibitory (antigrowth) signals	TGF- β 1	123	Survival	Overexpression correlated with poorer survival on univariate analysis only	$P = 0.0255$	[85]
	APC	57	Survival	High plasma levels correlated with poorer overall survival	$P = 0.0317$	[86]
		52	Survival	High plasma levels of methylation of APC associated with poorer survival	$P = 0.016$	[87]
	P21	30	Survival	Alteration in expression after chemotherapy correlated with better survival	$P = 0.011$	[88,89]
Evasion of programmed cell death (apoptosis)	P53	30	Survival	Alteration in expression after chemotherapy correlated with better survival	$P = 0.011$	[88]
	Bcl-2	35	Survival	Expression correlated with poorer survival	$P = 0.03$	[90]
	COX-2	100	T-stage, N-stage, tumor recurrence and survival	Higher levels expression correlated with: Higher T-stage, Higher N-stage, Increased risk of tumor recurrence Poor survival	$P = 0.008$ $P = 0.049$ $P = 0.01$ $P < 0.001$	[91]
		20	Survival	Strong staining correlated with poorer survival	$P = 0.03$	[92]
		145	Distant metastasis, local recurrence and survival	Strong staining correlated with: Distant metastasis Local recurrence Poorer survival	$P = 0.02$ $P = 0.05$ $P = 0.002$	[93]
		43	Survival	Activated NF- κ B predictive of: Poorer disease free survival Poorer overall survival	$P = 0.010$ $P = 0.015$	[94]
	NF- κ B	46	Survival	Higher telomere-length ratio shown to be an independent poor prognostic factor	$P < 0.02$	[95]
				Significant correlation between expression and: Poorer survival	$P < 0.01$	[96]
				Presence of angiolymphatic invasion More lymph node metastasis Higher tumor stage More distant metastasis	$P < 0.05$ $P < 0.01$ $P < 0.001$ $P < 0.01$	
Limitless replicative potential Sustained angiogenesis	Telomerase	46	Survival	Higher telomere-length ratio shown to be an independent poor prognostic factor	$P < 0.02$	[95]
	CD105	75	Survival, angiolymphatic invasion, lymph node metastasis and tumor stage and distant metastasis	Significant correlation between expression and: Poorer survival Presence of angiolymphatic invasion More lymph node metastasis Higher tumor stage More distant metastasis	$P < 0.01$ $P < 0.05$ $P < 0.01$ $P < 0.001$ $P < 0.01$	[96]
		75	Survival, angiolymphatic invasion, lymph node metastasis, stage of tumor and distant metastasis	Significant correlation between high expression and: Poorer survival Presence of angiolymphatic invasion More lymph node metastasis Higher stage of tumor More distant metastasis	$P < 0.01$ $P < 0.05$ $P < 0.01$ $P < 0.01$ $P < 0.01$	
	VEGF	75	Survival, angiolymphatic invasion, lymph node metastasis, stage of tumor and distant metastasis	Significant correlation between high expression and: Poorer survival Presence of angiolymphatic invasion More lymph node metastasis Higher stage of tumor More distant metastasis	$P < 0.01$ $P < 0.05$ $P < 0.01$ $P < 0.01$ $P < 0.01$	
Tissue invasion and metastasis	Cadherin	59	Survival	Reduced level correlated with poorer overall survival	HR: 3.3, $P = 0.05$	[80]
	uPA	54	Survival	High uPA correlated with poorer survival	$P = 0.0002$	[97]
	TIMP	24	Survival and disease stage	Reduction of expression correlated with poorer overall survival and higher disease stage	$P = 0.007$ $P = 0.046$	[98]
Others	Promoter hypermethylation	41	Survival and tumor recurrence	Earlier tumor recurrence and poorer overall survival if > 50% of gene profile methylated	$P = 0.05$	[99]
		84	Differentiation	Hypermethylation of MGMT (Methylated-DNA-protein-cysteine methyltransferase) gene correlated with: Higher tumor differentiation	$P = 0.0079$	[100]

EGFR: Epidermal growth factor receptor; Her2/neu: Human EGFR2; TGF: Transforming growth factor; APC: Adenomatosis polyposis coli; P21: Cyclin-dependent kinase inhibitor 1; Bcl-2: B-cell lymphoma 2; COX-2: Cyclooxygenase-2; NF- κ B: Nuclear factor- κ B; CD105: Endoglin; VEGF: Vascular endothelial growth factor; uPA: Urokinase-type plasminogen activator; TIMP: Tissue inhibitor of metalloproteinase.

specificity. Panels of biomarkers such as the eight-gene methylation panel or the Reid panel, which combine LOH at various loci and DNA content abnormalities to

predict progression, seem to provide the most accurate predictor of progression based on statistics. Unfortunately, the common theme in these panels of markers is

Table 3 Summary of the molecular signatures discovered by microarray technology and latest methods used to correlate molecular alterations and prognosis in patients with esophageal adenocarcinoma

Method	Sample size (n)	Outcome	Findings	Statistical significance	External validation	Ref.
Oligonucleotide cRNA microarray	75	Survival	A 4-gene signature prognosticated patients	$P = 0.0001$	Yes	[113]
	77	Lymphatic spread	Created a gene signature predicting lymph node metastasis	Argininosuccinate synthetase expression (ASS) ($P = 0.048$)	No	[114]
	19	Chemotherapy response	Unsupervised hierarchical clustering divided patients into 2 groups, one of which responded to preoperative chemotherapy	Not statistically significant	No	[115,116]
	47	Chemotherapy response	86 genes dysregulated Ephrin B3 expression associated with chemotherapy response, tumor grading and stage	$P < 0.001$	No	[117]
Oligonucleotide cDNA microarray	46	Chemotherapy response	Gene signature not predictive in adenocarcinoma of esophagus	Not statistically significant	No	[118]
Proteomic analysis	34	Chemotherapy response	HSP27 expression associated with response to chemotherapy	$P < 0.05$	No	[119]
Single nucleotide polymorphism	210	Survival and recurrence	5 polymorphisms in 3 genes associated with longer recurrence free survival and reduced recurrence	$P = 0.004$	No	[120]
microRNAs analysis	96	Survival	Low miR-375 levels associated with worse survival	$P = 0.002$	No	[121]
Multiplex ligation-dependent probe amplification	33	Survival	Patients with more than 12 chromosomal aberrations had a poorer outcome than patients with < 12	$P = 0.014$	No	[122]

that they are far too expensive to be applied in routine clinical use, and technical expertise is not available in all centers to utilize these panels of biomarkers. The issue of costs and practicality of biomarkers should be one of the principle considerations before research and resources are channeled into it.

Although traditional methods of identifying biomarkers in Barrett's esophagus and its transition to dysplasia and EA have helped greatly in the understanding of the disease process, new technologies to create molecular signatures have also helped by identifying many important biomarkers not previously thought to be involved in its pathogenesis. A few biomarkers identified from both traditional methods and new technological platforms have shown great potential in predicting the progression from Barrett's esophagus to EA. However, a concerted effort is still needed to validate these biomarkers or molecular signatures in independent, large-scale prospective cohorts and to develop inexpensive, practical assays to allow for clinical applicability. Realistically, this can only be achieved by a multicenter collaboration to tackle the challenges of the large amount of resources, scientific and clinical input required to advance the field of biomarkers in Barrett's esophagus. There are a few major collaborations in the United Kingdom to date, and they include the Chemoprevention of Premalignant Intestinal neoplasia trial (CHO-PIN) and Oesophageal Cancer Clinical and Molecular Stratification Study (OCCAMS). This is also mirrored in the international arena with Barrett's Esophagus and Adenocarcinoma Consortium (BEACON) and Asian Barrett's Consortium as two examples of collaborative work on Barrett's esophagus. These initiatives allow for the pooling of resources, expertise and knowledge between centers and allow for the recruitment of large numbers of patients that are necessary to advance the field of biomarkers in Barrett's esophagus and EA. Although each study

has a slightly different focus, much could be gained these collaborative efforts if a proportion of the resources and patient samples could be used to validate biomarkers in Barrett's or tumor samples.

Lastly, biomarkers should be seen as adjuncts to aid clinical management of patients with Barrett's esophagus and EA rather than in isolation in predicting the risk of progression, prognosis or response to therapy. As such, clinical factors in conjunction with biomarkers should be incorporated into a model that can accurately determine the desired outcome. Such models have been used in other cancers and diseases such as the MELD score for liver disease or the Nottingham prognostic index for breast cancer. Upon generation and validation of the model, it should then be rigorously validated in an independent large cohort of patients in a prospective fashion. In future, patients can then be risk stratified based on a score to determine the treatment strategy, hence individualizing treatment to improve patient care and outcome.

ACKNOWLEDGMENTS

We would like to thank Dr. Elizabeth Bird-Lieberman for her kind contributions of the scanned histology slides for Figure 1.

REFERENCES

- 1 **Playford RJ.** New British Society of Gastroenterology (BSG) guidelines for the diagnosis and management of Barrett's oesophagus. *Gut* 2006; **55**: 442
- 2 **Ronkainen J,** Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, Vieth M, Stolte M, Talley NJ, Agrés L. Prevalence of Barrett's esophagus in the general population: an endoscopic study. *Gastroenterology* 2005; **129**: 1825-1831
- 3 **Curvers WL,** ten Kate FJ, Krishnadath KK, Visser M, Elzer B, Baak LC, Bohmer C, Mallant-Hent RC, van Oijen A, Naber AH, Scholten P, Busch OR, Blaauwgeers HG, Meijer GA,

- Bergman JJ. Low-grade dysplasia in Barrett's esophagus: overdiagnosed and underestimated. *Am J Gastroenterol* 2010; **105**: 1523-1530
- 4 Wani S, Puli SR, Shaheen NJ, Westhoff B, Sleghria S, Bansal A, Rastogi A, Sayana H, Sharma P. Esophageal adenocarcinoma in Barrett's esophagus after endoscopic ablative therapy: a meta-analysis and systematic review. *Am J Gastroenterol* 2009; **104**: 502-513
- 5 Paull A, Trier JS, Dalton MD, Camp RC, Loeb P, Goyal RK. The histologic spectrum of Barrett's esophagus. *N Engl J Med* 1976; **295**: 476-480
- 6 Spechler SJ, Goyal RK. The columnar-lined esophagus, intestinal metaplasia, and Norman Barrett. *Gastroenterology* 1996; **110**: 614-621
- 7 Kelty CJ, Gough MD, Van Wyk Q, Stephenson TJ, Ackroyd R. Barrett's oesophagus: intestinal metaplasia is not essential for cancer risk. *Scand J Gastroenterol* 2007; **42**: 1271-1274
- 8 Wang KK, Sampliner RE. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. *Am J Gastroenterol* 2008; **103**: 788-797
- 9 Riddell RH, Odze RD. Definition of Barrett's esophagus: time for a rethink--is intestinal metaplasia dead? *Am J Gastroenterol* 2009; **104**: 2588-2594
- 10 Fitzgerald RC. Molecular basis of Barrett's oesophagus and oesophageal adenocarcinoma. *Gut* 2006; **55**: 1810-1820
- 11 van Sandick JW, van Lanschot JJ, Kuiken BW, Tytgat GN, Offerhaus GJ, Obertop H. Impact of endoscopic biopsy surveillance of Barrett's oesophagus on pathological stage and clinical outcome of Barrett's carcinoma. *Gut* 1998; **43**: 216-222
- 12 Wong T, Tian J, Nagar AB. Barrett's surveillance identifies patients with early esophageal adenocarcinoma. *Am J Med* 2010; **123**: 462-467
- 13 Schlansky B, Dimarino AJ Jr, Loren D, Infantolino A, Kowalski T, Cohen S. A survey of oesophageal cancer: pathology, stage and clinical presentation. *Aliment Pharmacol Ther* 2006; **23**: 587-593
- 14 Gerson LB, Shetler K, Triadafilopoulos G. Prevalence of Barrett's esophagus in asymptomatic individuals. *Gastroenterology* 2002; **123**: 461-467
- 15 Rex DK, Cummings OW, Shaw M, Cumings MD, Wong RK, Vasudeva RS, Dunne D, Rahmani EY, Helper DJ. Screening for Barrett's esophagus in colonoscopy patients with and without heartburn. *Gastroenterology* 2003; **125**: 1670-1677
- 16 Ward EM, Wolfsen HC, Achem SR, Loeb DS, Krishna M, Hemminger LL, DeVault KR. Barrett's esophagus is common in older men and women undergoing screening colonoscopy regardless of reflux symptoms. *Am J Gastroenterol* 2006; **101**: 12-17
- 17 Reid BJ, Haggitt RC, Rubin CE, Roth G, Surawicz CM, Van Belle G, Lewin K, Weinstein WM, Antonioli DA, Goldman H. Observer variation in the diagnosis of dysplasia in Barrett's esophagus. *Hum Pathol* 1988; **19**: 166-178
- 18 Montgomery E, Bronner MP, Goldblum JR, Greenson JK, Haber MM, Hart J, Lamps LW, Lauwers GY, Lazenby AJ, Lewin DN, Robert ME, Toledano AY, Shyr Y, Washington K. Reproducibility of the diagnosis of dysplasia in Barrett esophagus: a reaffirmation. *Hum Pathol* 2001; **32**: 368-378
- 19 Reid BJ, Li X, Galipeau PC, Vaughan TL. Barrett's oesophagus and oesophageal adenocarcinoma: time for a new synthesis. *Nat Rev Cancer* 2010; **10**: 87-101
- 20 Shaheen NJ, Crosby MA, Bozyski EM, Sandler RS. Is there publication bias in the reporting of cancer risk in Barrett's esophagus? *Gastroenterology* 2000; **119**: 333-338
- 21 Somerville M, Garside R, Pitt M, Stein K. Surveillance of Barrett's oesophagus: is it worthwhile? *Eur J Cancer* 2008; **44**: 588-599
- 22 Manne U, Srivastava RG, Srivastava S. Recent advances in biomarkers for cancer diagnosis and treatment. *Drug Discov Today* 2005; **10**: 965-976
- 23 Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, Winget M, Yasui Y. Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst* 2001; **93**: 1054-1061
- 24 McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. REporting recommendations for tumour MARKer prognostic studies (REMARK). *Br J Cancer* 2005; **93**: 387-391
- 25 Anderson NL, Anderson NG. The human plasma proteome: history, character, and diagnostic prospects. *Mol Cell Proteomics* 2002; **1**: 845-867
- 26 Srivastava S, Verma M, Henson DE. Biomarkers for early detection of colon cancer. *Clin Cancer Res* 2001; **7**: 1118-1126
- 27 Mitra AP, Bartsch CC, Cote RJ. Strategies for molecular expression profiling in bladder cancer. *Cancer Metastasis Rev* 2009; **28**: 317-326
- 28 Lao-Sirieix P, Boussioutas A, Kadri SR, O'Donovan M, Debiram I, Das M, Harihar L, Fitzgerald RC. Non-endoscopic screening biomarkers for Barrett's oesophagus: from microarray analysis to the clinic. *Gut* 2009; **58**: 1451-1459
- 29 Weston AP, Sharma P, Topalovski M, Richards R, Cherian R, Dixon A. Long-term follow-up of Barrett's high-grade dysplasia. *Am J Gastroenterol* 2000; **95**: 1888-1893
- 30 Overholt BF, Lightdale CJ, Wang KK, Canto MI, Burdick S, Haggitt RC, Bronner MP, Taylor SL, Grace MG, Depot M. 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**: 488-498
- 31 Reid BJ, Levine DS, Longton G, Blount PL, Rabinovitch PS. 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**: 1669-1676
- 32 Schnell TG, Sontag SJ, Chejfec G, Aranha G, Metz A, O'Connell S, Seidel UJ, Sonnenberg A. Long-term nonsurgical management of Barrett's esophagus with high-grade dysplasia. *Gastroenterology* 2001; **120**: 1607-1619
- 33 Galipeau PC, Li X, Blount PL, Maley CC, Sanchez CA, Odze RD, Ayub K, Rabinovitch PS, Vaughan TL, Reid BJ. NSAIDs modulate CDKN2A, TP53, and DNA content risk for progression to esophageal adenocarcinoma. *PLoS Med* 2007; **4**: e67
- 34 Murray L, Sedo A, Scott M, McManus D, Sloan JM, Hardie LJ, Forman D, Wild CP. TP53 and progression from Barrett's metaplasia to oesophageal adenocarcinoma in a UK population cohort. *Gut* 2006; **55**: 1390-1397
- 35 Weston AP, Banerjee SK, Sharma P, Tran TM, Richards R, Cherian R. p53 protein overexpression in low grade dysplasia (LGD) in Barrett's esophagus: immunohistochemical marker predictive of progression. *Am J Gastroenterol* 2001; **96**: 1355-1362
- 36 Sirieix PS, O'Donovan M, Brown J, Save V, Coleman N, Fitzgerald RC. Surface expression of minichromosome maintenance proteins provides a novel method for detecting patients at risk for developing adenocarcinoma in Barrett's esophagus. *Clin Cancer Res* 2003; **9**: 2560-2566
- 37 Lao-Sirieix P, Lovat L, Fitzgerald RC. Cyclin A immunocytology as a risk stratification tool for Barrett's esophagus surveillance. *Clin Cancer Res* 2007; **13**: 659-665
- 38 Schulmann K, Sterian A, Berki A, Yin J, Sato F, Xu Y, Olaru A, Wang S, Mori Y, Deacu E, Hamilton J, Kan T, Krasna MJ, Beer DG, Pepe MS, Abraham JM, Feng Z, Schmiegel W, Greenwald BD, Meltzer SJ. Inactivation of p16, RUNX3, and HPP1 occurs early in Barrett's-associated neoplastic progression and predicts progression risk. *Oncogene* 2005; **24**: 4138-4148
- 39 Jin Z, Cheng Y, Gu W, Zheng Y, Sato F, Mori Y, Olaru AV, Paun BC, Yang J, Kan T, Ito T, Hamilton JP, Selaru FM, Agarwal R, David S, Abraham JM, Wolfsen HC, Wallace MB, Shaheen NJ, Washington K, Wang J, Canto MI, Bhat-tacharya A, Nelson MA, Wagner PD, Romero Y, Wang KK,

- Feng Z, Sampliner RE, Meltzer SJ. A multicenter, double-blinded validation study of methylation biomarkers for progression prediction in Barrett's esophagus. *Cancer Res* 2009; **69**: 4112-4115
- 40 **Schlemper RJ**, Riddell RH, Kato Y, Borchard F, Cooper HS, Dawsey SM, Dixon MF, Fenoglio-Preiser CM, Fléjou JF, Geboes K, Hattori T, Hirota T, Itabashi M, Iwafuchi M, Iwashita A, Kim YI, Kirchner T, Klimpfinger M, Koike M, Lauwers GY, Lewin KJ, Oberhuber G, Offner F, Price AB, Rubio CA, Shimizu M, Shimoda T, Sipponen P, Solcia E, Stolte M, Watanabe H, Yamabe H. The Vienna classification of gastrointestinal epithelial neoplasia. *Gut* 2000; **47**: 251-255
 - 41 **Pech O**, Vieth M, Schmitz D, Gossner L, May A, Seitz G, Stolte M, Ell C. Conclusions from the histological diagnosis of low-grade intraepithelial neoplasia in Barrett's oesophagus. *Scand J Gastroenterol* 2007; **42**: 682-688
 - 42 **Jankowski JA**, Odze RD. Biomarkers in gastroenterology: between hope and hype comes histopathology. *Am J Gastroenterol* 2009; **104**: 1093-1096
 - 43 **Rabinovitch PS**, Longton G, Blount PL, Levine DS, Reid BJ. Predictors of progression in Barrett's esophagus III: baseline flow cytometric variables. *Am J Gastroenterol* 2001; **96**: 3071-3083
 - 44 **Galipeau PC**, Cowan DS, Sanchez CA, Barrett MT, Emond MJ, Levine DS, Rabinovitch PS, Reid BJ. 17p (p53) allelic losses, 4N (G2/tetraploid) populations, and progression to aneuploidy in Barrett's esophagus. *Proc Natl Acad Sci USA* 1996; **93**: 7081-7084
 - 45 **Fang M**, Lew E, Klein M, Sebo T, Su Y, Goyal R. DNA abnormalities as marker of risk for progression of Barrett's esophagus to adenocarcinoma: image cytometric DNA analysis in formalin-fixed tissues. *Am J Gastroenterol* 2004; **99**: 1887-1894
 - 46 **Wong DJ**, Paulson TG, Prevo LJ, Galipeau PC, Longton G, Blount PL, Reid BJ. p16(INK4a) lesions are common, early abnormalities that undergo clonal expansion in Barrett's metaplastic epithelium. *Cancer Res* 2001; **61**: 8284-8289
 - 47 **Leedham SJ**, Preston SL, McDonald SA, Elia G, Bhandari P, Poller D, Harrison R, Novelli MR, Jankowski JA, Wright NA. Individual crypt genetic heterogeneity and the origin of metaplastic glandular epithelium in human Barrett's oesophagus. *Gut* 2008; **57**: 1041-1048
 - 48 **Reid BJ**, Prevo LJ, Galipeau PC, Sanchez CA, Longton G, Levine DS, Blount PL, Rabinovitch PS. Predictors of progression in Barrett's esophagus II: baseline 17p (p53) loss of heterozygosity identifies a patient subset at increased risk for neoplastic progression. *Am J Gastroenterol* 2001; **96**: 2839-2848
 - 49 **Vogt N**, Schönegg R, Gschossmann JM, Borovicka J. Benefit of baseline cytometry for surveillance of patients with Barrett's esophagus. *Surg Endosc* 2010; **24**: 1144-1150
 - 50 **Huang Q**, Yu C, Klein M, Fang J, Goyal RK. DNA index determination with Automated Cellular Imaging System (ACIS) in Barrett's esophagus: comparison with CAS 200. *BMC Clin Pathol* 2005; **5**: 7
 - 51 **Yu C**, Zhang X, Huang Q, Klein M, Goyal RK. High-fidelity DNA histograms in neoplastic progression in Barrett's esophagus. *Lab Invest* 2007; **87**: 466-472
 - 52 **Li X**, Galipeau PC, Sanchez CA, Blount PL, Maley CC, Arnaudo J, Peiffer DA, Pokholok D, Gunderson KL, Reid BJ. Single nucleotide polymorphism-based genome-wide chromosome copy change, loss of heterozygosity, and aneuploidy in Barrett's esophagus neoplastic progression. *Cancer Prev Res (Phila Pa)* 2008; **1**: 413-423
 - 53 **Paulson TG**, Galipeau PC, Reid BJ. Loss of heterozygosity analysis using whole genome amplification, cell sorting, and fluorescence-based PCR. *Genome Res* 1999; **9**: 482-491
 - 54 **Wongsurawat VJ**, Finley JC, Galipeau PC, Sanchez CA, Maley CC, Li X, Blount PL, Odze RD, Rabinovitch PS, Reid BJ. Genetic mechanisms of TP53 loss of heterozygosity in Barrett's esophagus: implications for biomarker validation. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 509-516
 - 55 **Hong MK**, Laskin WB, Herman BE, Johnston MH, Vargo JJ, Steinberg SM, Allegra CJ, Johnston PG. Expansion of the Ki-67 proliferative compartment correlates with degree of dysplasia in Barrett's esophagus. *Cancer* 1995; **75**: 423-429
 - 56 **Going JJ**, Keith WN, Neilson L, Stoeber K, Stuart RC, Williams GH. Aberrant expression of minichromosome maintenance proteins 2 and 5, and Ki-67 in dysplastic squamous oesophageal epithelium and Barrett's mucosa. *Gut* 2002; **50**: 373-377
 - 57 **Trudgill NJ**, Suvarna SK, Royds JA, Riley SA. Cell cycle regulation in patients with intestinal metaplasia at the gastro-oesophageal junction. *Mol Pathol* 2003; **56**: 313-317
 - 58 **Bani-Hani K**, Martin IG, Hardie LJ, Mapstone N, Briggs JA, Forman D, Wild CP. Prospective study of cyclin D1 overexpression in Barrett's esophagus: association with increased risk of adenocarcinoma. *J Natl Cancer Inst* 2000; **92**: 1316-1321
 - 59 **Eads CA**, Lord RV, Kurumboor SK, Wickramasinghe K, Skinner ML, Long TI, Peters JH, DeMeester TR, Danenberg KD, Danenberg PV, Laird PW, Skinner KA. Fields of aberrant CpG island hypermethylation in Barrett's esophagus and associated adenocarcinoma. *Cancer Res* 2000; **60**: 5021-5026
 - 60 **Jin Z**, Cheng Y, Gu W, Zheng Y, Sato F, Mori Y, Oлару AV, Paun BC, Yang J, Kan T, Ito T, Hamilton JP, Selaru FM, Agarwal R, David S, Abraham JM, Wolfsen HC, Wallace MB, Shaheen NJ, Washington K, Wang J, Canto MI, Bhattacharyya A, Nelson MA, Wagner PD, Romero Y, Wang KK, Feng Z, Sampliner RE, Meltzer SJ. A multicenter, double-blinded validation study of methylation biomarkers for progression prediction in Barrett's esophagus. *Cancer Res* 2009; **69**: 4112-4115
 - 61 **Wang JS**, Guo M, Montgomery EA, Thompson RE, Cosby H, Hicks L, Wang S, Herman JG, Canto MI. DNA promoter hypermethylation of p16 and APC predicts neoplastic progression in Barrett's esophagus. *Am J Gastroenterol* 2009; **104**: 2153-2160
 - 62 **Eads CA**, Lord RV, Wickramasinghe K, Long TI, Kurumboor SK, Bernstein L, Peters JH, DeMeester SR, DeMeester TR, Skinner KA, Laird PW. Epigenetic patterns in the progression of esophageal adenocarcinoma. *Cancer Res* 2001; **61**: 3410-3418
 - 63 **Clark SJ**, Harrison J, Paul CL, Frommer M. High sensitivity mapping of methylated cytosines. *Nucleic Acids Res* 1994; **22**: 2990-2997
 - 64 **Weber M**, Davies JJ, Wittig D, Oakeley EJ, Haase M, Lam WL, Schübeler D. Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. *Nat Genet* 2005; **37**: 853-862
 - 65 **Huang TH**, Laux DE, Hamlin BC, Tran P, Tran H, Lubahn DB. Identification of DNA methylation markers for human breast carcinomas using the methylation-sensitive restriction fingerprinting technique. *Cancer Res* 1997; **57**: 1030-1034
 - 66 **Khulan B**, Thompson RF, Ye K, Fazzari MJ, Suzuki M, Stasiak E, Figueroa ME, Glass JL, Chen Q, Montagna C, Hatchwell E, Selzer RR, Richmond TA, Green RD, Melnick A, Grealley JM. Comparative isoschizomer profiling of cytosine methylation: the HELP assay. *Genome Res* 2006; **16**: 1046-1055
 - 67 **Frigola J**, Ribas M, Risques RA, Peinado MA. Methylome profiling of cancer cells by amplification of inter-methylated sites (AIMS). *Nucleic Acids Res* 2002; **30**: e28
 - 68 **Ushijima T**, Morimura K, Hosoya Y, Okonogi H, Tatematsu M, Sugimura T, Nagao M. Establishment of methylation-sensitive-representational difference analysis and isolation of hypo- and hypermethylated genomic fragments in mouse liver tumors. *Proc Natl Acad Sci USA* 1997; **94**: 2284-2289
 - 69 **Hatada I**, Hayashizaki Y, Hirotsune S, Komatsubara H, Mukai T. A genomic scanning method for higher organisms using restriction sites as landmarks. *Proc Natl Acad Sci USA* 1991; **88**: 9523-9527

- 70 **Bibikova M**, Lin Z, Zhou L, Chudin E, Garcia EW, Wu B, Doucet D, Thomas NJ, Wang Y, Vollmer E, Goldmann T, Seifart C, Jiang W, Barker DL, Chee MS, Floros J, Fan JB. High-throughput DNA methylation profiling using universal bead arrays. *Genome Res* 2006; **16**: 383-393
- 71 **Ibanez de Caceres I**, Dulaimi E, Hoffman AM, Al-Saleem T, Uzzo RG, Cairns P. Identification of novel target genes by an epigenetic reactivation screen of renal cancer. *Cancer Res* 2006; **66**: 5021-5028
- 72 **Laird PW**. Principles and challenges of genome-wide DNA methylation analysis. *Nat Rev Genet* 2010; **11**: 191-203
- 73 **CancerStats**. Cancer Research UK, 2009. Accessed on 25/11/2009. Available from: URL: <http://info.cancerresearchuk.org/cancerstats/>
- 74 **Edge SB**, Compton CC, Fritz AG, Greene FL, Trotti A. AJCC Cancer Staging Manual. New York, NY: Springer, 2010
- 75 **Hanahan D**, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**: 57-70
- 76 **Lagarde SM**, ten Kate FJ, Richel DJ, Offerhaus GJ, van Lanschot JJ. Molecular prognostic factors in adenocarcinoma of the esophagus and gastroesophageal junction. *Ann Surg Oncol* 2007; **14**: 977-991
- 77 **Izzo JG**, Wu TT, Wu X, Ensor J, Luthra R, Pan J, Correa A, Swisher SG, Chao CK, Hittelman WN, Ajani JA. Cyclin D1 guanine/adenine 870 polymorphism with altered protein expression is associated with genomic instability and aggressive clinical biology of esophageal adenocarcinoma. *J Clin Oncol* 2007; **25**: 698-707
- 78 **Wang KL**, Wu TT, Choi IS, Wang H, Resetkova E, Correa AM, Hofstetter WL, Swisher SG, Ajani JA, Rashid A, Albaracin CT. Expression of epidermal growth factor receptor in esophageal and esophagogastric junction adenocarcinomas: association with poor outcome. *Cancer* 2007; **109**: 658-667
- 79 **Langer R**, Von Rahden BH, Nahrig J, Von Weyhern C, Reiter R, Feith M, Stein HJ, Siewert JR, Höfler H, Sarbia M. Prognostic significance of expression patterns of c-erbB-2, p53, p16INK4A, p27KIP1, cyclin D1 and epidermal growth factor receptor in oesophageal adenocarcinoma: a tissue microarray study. *J Clin Pathol* 2006; **59**: 631-634
- 80 **Falkenback D**, Nilbert M, Oberg S, Johansson J. Prognostic value of cell adhesion in esophageal adenocarcinomas. *Dis Esophagus* 2008; **21**: 97-102
- 81 **Brien TP**, Odze RD, Sheehan CE, McKenna BJ, Ross JS. HER-2/neu gene amplification by FISH predicts poor survival in Barrett's esophagus-associated adenocarcinoma. *Hum Pathol* 2000; **31**: 35-39
- 82 **Ross JS**, McKenna BJ. The HER-2/neu oncogene in tumors of the gastrointestinal tract. *Cancer Invest* 2001; **19**: 554-568
- 83 **Aloia TA**, Harpole DH Jr, Reed CE, Allegra C, Moore MB, Herndon JE 2nd, D'Amico TA. Tumor marker expression is predictive of survival in patients with esophageal cancer. *Ann Thorac Surg* 2001; **72**: 859-866
- 84 **D'Errico A**, Barozzi C, Fiorentino M, Carella R, Di Simone M, Ferruzzi L, Mattioli S, Grigioni WF. Role and new perspectives of transforming growth factor-alpha (TGF-alpha) in adenocarcinoma of the gastro-oesophageal junction. *Br J Cancer* 2000; **82**: 865-870
- 85 **von Rahden BH**, Stein HJ, Feith M, Pühringer F, Theisen J, Siewert JR, Sarbia M. Overexpression of TGF-beta1 in esophageal (Barrett's) adenocarcinoma is associated with advanced stage of disease and poor prognosis. *Mol Carcinog* 2006; **45**: 786-794
- 86 **Fukuchi M**, Miyazaki T, Fukai Y, Nakajima M, Sohda M, Masuda N, Manda R, Tsukada K, Kato H, Kuwano H. Plasma level of transforming growth factor beta1 measured from the azygos vein predicts prognosis in patients with esophageal cancer. *Clin Cancer Res* 2004; **10**: 2738-2741
- 87 **Kawakami K**, Brabender J, Lord RV, Groshen S, Greenwald BD, Krasna MJ, Yin J, Fleisher AS, Abraham JM, Beer DG, Sidransky D, Huss HT, Demeester TR, Eads C, Laird PW, Ilson DH, Kelsen DP, Harpole D, Moore MB, Danenberg KD, Danenberg PV, Meltzer SJ. Hypermethylated APC DNA in plasma and prognosis of patients with esophageal adenocarcinoma. *J Natl Cancer Inst* 2000; **92**: 1805-1811
- 88 **Heeren PA**, Kloppenberg FW, Hollema H, Mulder NH, Nap RE, Plukker JT. Predictive effect of p53 and p21 alteration on chemotherapy response and survival in locally advanced adenocarcinoma of the esophagus. *Anticancer Res* 2004; **24**: 2579-2583
- 89 **Nakashima S**, Natsugoe S, Matsumoto M, Kijima F, Takebayashi Y, Okumura H, Shimada M, Nakano S, Kusano C, Baba M, Takao S, Aikou T. Expression of p53 and p21 is useful for the prediction of preoperative chemotherapeutic effects in esophageal carcinoma. *Anticancer Res* 2000; **20**: 1933-1937
- 90 **Raouf AA**, Evoy DA, Carton E, Mulligan E, Griffin MM, Reynolds JV. Loss of Bcl-2 expression in Barrett's dysplasia and adenocarcinoma is associated with tumor progression and worse survival but not with response to neoadjuvant chemoradiation. *Dis Esophagus* 2003; **16**: 17-23
- 91 **Bhandari P**, Bateman AC, Mehta RL, Stacey BS, Johnson P, Cree IA, Di Nicolantonio F, Patel P. Prognostic significance of cyclooxygenase-2 (COX-2) expression in patients with surgically resectable adenocarcinoma of the oesophagus. *BMC Cancer* 2006; **6**: 134
- 92 **France M**, Drew PA, Dodd T, Watson DI. Cyclo-oxygenase-2 expression in esophageal adenocarcinoma as a determinant of clinical outcome following esophagectomy. *Dis Esophagus* 2004; **17**: 136-140
- 93 **Buskens CJ**, Van Rees BP, Sivula A, Reitsma JB, Haglund C, Bosma PJ, Offerhaus GJ, Van Lanschot JJ, Ristimäki A. Prognostic significance of elevated cyclooxygenase 2 expression in patients with adenocarcinoma of the esophagus. *Gastroenterology* 2002; **122**: 1800-1807
- 94 **Izzo JG**, Malhotra U, Wu TT, Ensor J, Luthra R, Lee JH, Swisher SG, Liao Z, Chao KS, Hittelman WN, Aggarwal BB, Ajani JA. Association of activated transcription factor nuclear factor kappaB with chemoradiation resistance and poor outcome in esophageal carcinoma. *J Clin Oncol* 2006; **24**: 748-754
- 95 **Gertler R**, Doll D, Maak M, Feith M, Rosenberg R. Telomere length and telomerase subunits as diagnostic and prognostic biomarkers in Barrett carcinoma. *Cancer* 2008; **112**: 2173-2180
- 96 **Saad RS**, El-Gohary Y, Memari E, Liu YL, Silverman JF. Endoglin (CD105) and vascular endothelial growth factor as prognostic markers in esophageal adenocarcinoma. *Hum Pathol* 2005; **36**: 955-961
- 97 **Nekarda H**, Schlegel P, Schmitt M, Stark M, Mueller JD, Fink U, Siewert JR. Strong prognostic impact of tumor-associated urokinase-type plasminogen activator in completely resected adenocarcinoma of the esophagus. *Clin Cancer Res* 1998; **4**: 1755-1763
- 98 **Darnton SJ**, Hardie LJ, Muc RS, Wild CP, Casson AG. Tissue inhibitor of metalloproteinase-3 (TIMP-3) gene is methylated in the development of esophageal adenocarcinoma: loss of expression correlates with poor prognosis. *Int J Cancer* 2005; **115**: 351-358
- 99 **Brock MV**, Gou M, Akiyama Y, Muller A, Wu TT, Montgomery E, Deasel M, Germonpré P, Rubinson L, Heitmiller RF, Yang SC, Forastiere AA, Baylin SB, Herman JG. Prognostic importance of promoter hypermethylation of multiple genes in esophageal adenocarcinoma. *Clin Cancer Res* 2003; **9**: 2912-2919
- 100 **Baumann S**, Keller G, Pühringer F, Napieralski R, Feith M, Langer R, Höfler H, Stein HJ, Sarbia M. The prognostic impact of O6-Methylguanine-DNA Methyltransferase (MGMT) promoter hypermethylation in esophageal adenocarcinoma. *Int J Cancer* 2006; **119**: 264-268
- 101 **van't Veer LJ**, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen

- AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernardis R, Friend SH. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002; **415**: 530-536
- 102 **Fan C**, Oh DS, Wessels L, Weigelt B, Nuyten DS, Nobel AB, van't Veer LJ, Perou CM. Concordance among gene-expression-based predictors for breast cancer. *N Engl J Med* 2006; **355**: 560-569
 - 103 **Karlsson E**, Delle U, Danielsson A, Olsson B, Abel F, Karlsson P, Helou K. Gene expression variation to predict 10-year survival in lymph-node-negative breast cancer. *BMC Cancer* 2008; **8**: 254
 - 104 **Selaru FM**, Zou T, Xu Y, Shustova V, Yin J, Mori Y, Sato F, Wang S, Olaru A, Shibata D, Greenwald BD, Krasna MJ, Abraham JM, Meltzer SJ. Global gene expression profiling in Barrett's esophagus and esophageal cancer: a comparative analysis using cDNA microarrays. *Oncogene* 2002; **21**: 475-478
 - 105 **Greenawalt DM**, Duong C, Smyth GK, Ciavarella ML, Thompson NJ, Tiang T, Murray WK, Thomas RJ, Phillips WA. Gene expression profiling of esophageal cancer: comparative analysis of Barrett's esophagus, adenocarcinoma, and squamous cell carcinoma. *Int J Cancer* 2007; **120**: 1914-1921
 - 106 **Ntzani EE**, Ioannidis JP. Predictive ability of DNA microarrays for cancer outcomes and correlates: an empirical assessment. *Lancet* 2003; **362**: 1439-1444
 - 107 **Michiels S**, Koscielny S, Hill C. Prediction of cancer outcome with microarrays: a multiple random validation strategy. *Lancet* 2005; **365**: 488-492
 - 108 **Abu-Hanna A**, Lucas PJ. Prognostic models in medicine. AI and statistical approaches. *Methods Inf Med* 2001; **40**: 1-5
 - 109 **Royston P**, Moons KG, Altman DG, Vergouwe Y. Prognosis and prognostic research: Developing a prognostic model. *BMJ* 2009; **338**: b604
 - 110 **Moons KG**, Royston P, Vergouwe Y, Grobbee DE, Altman DG. Prognosis and prognostic research: what, why, and how? *BMJ* 2009; **338**: b375
 - 111 **Moons KG**, Altman DG, Vergouwe Y, Royston P. Prognosis and prognostic research: application and impact of prognostic models in clinical practice. *BMJ* 2009; **338**: b606
 - 112 **Altman DG**, Vergouwe Y, Royston P, Moons KG. Prognosis and prognostic research: validating a prognostic model. *BMJ* 2009; **338**: b605
 - 113 **Peters CJ**, Rees JR, Hardwick RH, Hardwick JS, Vowler SL, Ong CA, Zhang C, Save V, O'Donovan M, Rassl D, Alderson D, Caldas C, Fitzgerald RC. A 4-Gene Signature Predicts Survival of Patients With Resected Adenocarcinoma of the Esophagus, Junction, and Gastric Cardia. *Gastroenterology* 2010; Epub ahead of print
 - 114 **Lagarde SM**, Ver Loren van Themaat PE, Moerland PD, Gilhuijs-Pederson LA, Ten Kate FJ, Reitsma PH, van Kampen AH, Zwinderman AH, Baas F, van Lanschot JJ. Analysis of gene expression identifies differentially expressed genes and pathways associated with lymphatic dissemination in patients with adenocarcinoma of the esophagus. *Ann Surg Oncol* 2008; **15**: 3459-3470
 - 115 **Luthra MG**, Ajani JA, Izzo J, Ensor J, Wu TT, Rashid A, Zhang L, Phan A, Fukami N, Luthra R. Decreased expression of gene cluster at chromosome 1q21 defines molecular subgroups of chemoradiotherapy response in esophageal cancers. *Clin Cancer Res* 2007; **13**: 912-919
 - 116 **Luthra R**, Wu TT, Luthra MG, Izzo J, Lopez-Alvarez E, Zhang L, Bailey J, Lee JH, Bresalier R, Rashid A, Swisher SG, Ajani JA. Gene expression profiling of localized esophageal carcinomas: association with pathologic response to preoperative chemoradiation. *J Clin Oncol* 2006; **24**: 259-267
 - 117 **Schauer M**, Janssen KP, Rimkus C, Raggi M, Feith M, Friess H, Theisen J. Microarray-based response prediction in esophageal adenocarcinoma. *Clin Cancer Res* 2010; **16**: 330-337
 - 118 **Duong C**, Greenawalt DM, Kowalczyk A, Ciavarella ML, Raskutti G, Murray WK, Phillips WA, Thomas RJ. Pre-treatment gene expression profiles can be used to predict response to neoadjuvant chemoradiotherapy in esophageal cancer. *Ann Surg Oncol* 2007; **14**: 3602-3609
 - 119 **Langer R**, Ott K, Specht K, Becker K, Lordick F, Burian M, Herrmann K, Schratzenholz A, Cahill MA, Schwaiger M, Hofler H, Wester HJ. Protein expression profiling in esophageal adenocarcinoma patients indicates association of heat-shock protein 27 expression and chemotherapy response. *Clin Cancer Res* 2008; **14**: 8279-8287
 - 120 **Wu X**, Gu J, Wu TT, Swisher SG, Liao Z, Correa AM, Liu J, Etzel CJ, Amos CI, Huang M, Chiang SS, Milas L, Hittelman WN, Ajani JA. Genetic variations in radiation and chemotherapy drug action pathways predict clinical outcomes in esophageal cancer. *J Clin Oncol* 2006; **24**: 3789-3798
 - 121 **Mathé EA**, Nguyen GH, Bowman ED, Zhao Y, Budhu A, Schetter AJ, Braun R, Reimers M, Kumamoto K, Hughes D, Altorki NK, Casson AG, Liu CG, Wang XW, Yanaihara N, Hagiwara N, Dannenberg AJ, Miyashita M, Croce CM, Harris CC. MicroRNA expression in squamous cell carcinoma and adenocarcinoma of the esophagus: associations with survival. *Clin Cancer Res* 2009; **15**: 6192-6200
 - 122 **Pasello G**, Agata S, Bonaldi L, Corradin A, Montagna M, Zamarchi R, Parenti A, Cagol M, Zaninotto G, Ruol A, Ancona E, Amadori A, Saggioro D. DNA copy number alterations correlate with survival of esophageal adenocarcinoma patients. *Mod Pathol* 2009; **22**: 58-65

S- Editor Wang JL L- Editor Kerr C E- Editor Ma WH