

## Clinical biomarkers in esophageal adenocarcinoma

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### TABLE CONTENTS

1. Abstract
2. Introduction
3. Methods
4. Biomarkers
5. Molecular Biology
6. Endoscopic Biomarkers and Tissue Biomarkers
7. Serum and Urine Biomarkers
8. Predictive Biomarkers
9. Conclusion
10. Acknowledgments
11. References

## 1. ABSTRACT

This review describes genetic and molecular changes related to adenocarcinoma of the esophagus and gastroesophageal junction (GEJ) with emphasis on prognostic value and possibilities for targeted therapy in clinical setting. The progression of Barrett's esophagus to adenocarcinoma has been the focus of particular scrutiny, and a number of potential tissue and serum-based disease biomarkers have emerged. Tissue biomarkers allowing risk stratification of Barrett's are reviewed as well as strategies currently being used to discover novel biomarkers that will facilitate the early detection of esophageal adenocarcinoma.

## 2. INTRODUCTION

The incidence of adenocarcinoma of the esophagus has risen steadily in both the United States and in Europe over the last two to three decades, whereas the incidence of esophageal squamous carcinoma has remained relatively static (1, 2). Esophageal adenocarcinoma is frequently accompanied by Barrett's esophagus (BE), a metaplastic condition in which the squamous epithelium lining the lower esophagus is replaced by columnar epithelium, often of a specialized intestinal type. There has also been an apparent increase in the incidence of Barrett's metaplasia, but because this has paralleled the increasing

## Biomarkers in esophageal adenocarcinoma

use of upper gastrointestinal endoscopy, it is not clear if this increase is real or artifactual (3).

The morbidity and mortality associated with the various forms of esophagectomy remain high. Neo-adjuvant chemo- and radiotherapy may have a role in shrinking bulky T3 cancers, improving the chances of complete resection, and providing a survival advantage (4).

Although esophageal adenocarcinoma is frequently accompanied by Barrett's metaplasia, only approximately 5% of patients who present with esophageal adenocarcinoma have an antecedent diagnosis of Barrett's metaplasia (3, 5, 6).

BE predisposes patients to esophageal adenocarcinoma, a cancer with one of the fastest rising incidence rates over the past decade and a highly lethal malignancy once it is symptomatic (7, 8, 11). It is believed that esophageal adenocarcinoma arises as the final step of a postulated sequential change in the metaplastic epithelium, progressing from low-grade dysplasia (LGD), to high-grade dysplasia (HGD), and finally carcinoma. HGD on histologic samples has been used as the most reliable clinical biomarker of potential carcinogenesis, with studies reporting variable rates of progression to esophageal carcinoma (range, 16%–59%) (9-11).

Adenocarcinoma of the distal esophagus and gastroesophageal junction (GEJ) is an aggressive disease with early lymphatic and hematogenous dissemination. To produce a tumor metastasis (lymphatic as well as hematogenous), tumor cells must complete a multistep progression through a series of sequential and selective events. The metastatic process consists of tumor cell detachment, local invasion, (lymph) angiogenesis and survival in the circulation, adhesion to endothelial cells, extravasation and regrowth in different organs. In each step, causative molecules have been identified: these include cell adhesion molecules, various growth factors, matrix degradation enzymes and motility factors (12). A recent concept is the so-called epithelial–mesenchymal transition. This is an important process during development by which epithelial cells acquire mesenchymal, fibroblast-like properties and show reduced intercellular adhesion and increased motility, endowing the incipient cancer cell with invasive and metastatic properties (13).

Therefore, this review describes the recent advances in our understanding of genetic and molecular changes related to adenocarcinoma of the distal esophagus and GEJ with special emphasis on their specific prognostic value and possibilities for (future) targeted therapy in the clinical setting.

### 3. METHODS

A review of the literature concerning esophageal adenocarcinoma was performed. This review focuses on genetic and molecular changes as prognostic factors in adenocarcinoma of the distal esophagus and GEJ.

## 4. BIOMARKERS

A biomarker may be defined as a characteristic that is measured or evaluated as an indicator of pathological processes or a response to a therapeutic intervention. An ideal biomarker of malignancy will show variation in expression associated with the process of neoplastic transformation and will be detectable early in a premalignant phase. The discovery and evaluation of cancer biomarkers represents a very complex task necessitating multidisciplinary collaboration between epidemiologists, basic scientists, clinicians, and industry. The National Cancer Institute has recently formed the Early Detection Research Network (14) to facilitate this process, and this group has suggested that the process may be divided into five phases analogous to the clinical trial structure used in testing new drugs (15). Such a structure also helps in the evaluation of published biomarker studies.

## 5. MOLECULAR BIOLOGY

Several molecular alterations have been reported in Barrett esophagus and are implicated in the molecular pathogenesis of esophageal adenocarcinoma (16-19). Many such biomarkers have been proposed to have potential clinical application, in areas such as molecular diagnosis for early detection, to predict risk for disease progression in endoscopic surveillance programs, for staging and prognosis, to predict chemosensitivity, as intermediate biomarkers in chemoprevention studies, and as novel targets for anticancer therapies. However, relatively few will ultimately prove to be clinically useful, and the introduction of tumor markers into clinical practice has been poorly controlled (20).

To facilitate the translation of recent advances in basic science into clinical practice, the National Cancer Institute Early Detection Research Network (NCI-EDRN) proposed five phases to validate novel biomarkers used in screening and surveillance for the early detection of cancer (21). As these were developed as a conceptual framework for coordinating biomarker research, not all biomarkers need to progress consecutively through each of the following five phases before recommendations are made as to potential clinical application.

Briefly, the aim of phase 1, or preclinical exploratory studies, is to identify novel biomarkers in tumor tissues, using matched histologically normal tissues for comparison. Biomarkers identified by such phase 1 studies, considered to be potentially useful in clinical practice, are subject to phase 2 studies, primarily to validate assays and to estimate true and false-positive rates. Secondary aims of phase 2 are to optimize assay conditions, to compare assay techniques, and to explore associations between a biomarker and selected clinicopathologic factors in patients with cancer (eg, tumor histology, grade, etc) and normal controls (eg, gender, age, smoking history, etc). Phase 3 are retrospective longitudinal repository studies, utilizing banked tissues to evaluate the capacity of a biomarker to detect preclinical disease, and to define criteria for a positive screening test in

## Biomarkers in esophageal adenocarcinoma

preparation for phase 4. The ability of a biomarker to predict disease is determined by prospective screening studies (phase 4), which should also consider potential benefits of early detection, the feasibility, and costs of implementing a screening program.

The final phase (cancer control studies; phase 5) addresses whether biomarker-based screening will reduce cancer mortality. To date, no potential biomarker associated with Barrett esophagus has been evaluated in a phase 5 study. As several biomarkers have been identified in Barrett esophagus, and many implicated in disease progression, the reader is referred to recent reviews summarizing the results of selected phase 1 and 2 studies (16-19). The following sections will therefore summarize the results of a limited number of phase 3 and 4 studies that have evaluated selected biomarkers identified as having potential clinical application in the management of BE.

### 6. ENDOSCOPIC BIOMARKERS AND TISSUE BIOMARKERS

Most esophageal adenocarcinomas are diagnosed by endoscopy and biopsy. It will be apparent that there is often considerable tissue heterogeneity in the lower esophagus harboring an occult early cancer.

The diagnosis of early lesions may be difficult, because the endoscopist may not be able to recognize dysplastic areas of columnar mucosa or early cancer. Rigorous, systematic biopsy protocols may be able to distinguish between early invasive adenocarcinoma and high-grade dysplasia (22), but in many centers where such protocols are not rigidly adhered to, representative sampling remains a significant issue.

It has been suggested that endoscopically visible ulceration of the mucosa is a useful biomarker of malignancy (23). Endoscopic ultrasound has emerged as a technique for the preoperative assessment of T and N staging. Endoscopic ultrasound with a high frequency probe can also detect areas of mucosal thickening and therefore assist in the detection of some early lesions (24). Another approach has been to use methylene blue staining (25) or fluorophores such as 5-aminolevulinic acid (26) to visualize dysplastic or neoplastic tissue more readily at endoscopy.

Tissue biomarkers that have been evaluated by immunohistochemistry include cyclin D1, p53, and markers of cell proliferation. It has been recognized for some time that the cell cycle is dysregulated in dysplastic Barrett's mucosa with increased Ki67-labeling indices. Moreover, there is evidence of loss of spatial organization, with abnormal expression of Ki67 on the surface epithelium in HGD (27).

Immunohistochemical detection of p53 shows a higher fraction of positively staining adenocarcinomas (87%) compared with dysplastic (9–55%) or metaplastic (0%) mucosa, and frequently there is evidence of topographical colocalization of the positive staining with

dysplastic change in biopsies (28). It has been proposed that the combination of p53 protein expression and disordered proliferative architecture may be used as an objective biomarker to assist in the recognition and diagnosis of dysplastic change (29, 30). Tissue biomarkers may also be used directly to stratify the risk of progression. It has been reported that p53 protein expression colocalized to low-grade dysplasia conferred an increased risk of progression to multifocal high-grade dysplasia or adenocarcinoma (31, 32). Cyclin D1 expression in nondysplastic Barrett's has also been associated with an increased risk of progression to adenocarcinoma, but in one phase 3 case-control study, p53 protein expression did not confer an increased risk (33), and in fact, 69% of the patients progressing to cancer had negative p53 immunostaining.

Brush cytology has the potential to sample tissues more widely than endoscopic biopsy. Improved methods of cytological specimen preparation and immunocytochemistry using monoclonal antibodies to novel tissue biomarkers (34) may yet lead to a useful role for cytology.

### 7. SERUM AND URINE BIOMARKERS

Only a minority of patients presenting with esophageal adenocarcinoma have an antecedent biopsy diagnosis of Barrett's (5), and therefore, improved risk stratification in Barrett's is unlikely to result in significant reductions in mortality from esophageal adenocarcinoma at a population level. The development of robust biomarker assays applicable to blood or urine samples might assist in the stratification of risk in patients with symptoms of gastro-oesophageal reflux disease and the selection of patients for endoscopy of the upper gastro-intestinal tract. Villin has been found in the serum of about 50% of colon cancer patients and represents a useful marker to detect cancer recurrence after tumor resection (35).

Autoantibodies to villin were also detected in 80% of patients. Although the highest level of autoantibodies to villin was present in the cancer patients, these autoantibodies were also found in patients with inflammatory bowel disease and in some controls as well. The antigenicity of villin may result from the exposure of the villin protein, which is normally intracellular, after cell lysis occurring spontaneously in tumors or secondary to inflammation.

Determination of serum villin levels or detection of villin autoantibodies may assist in the identification of a subset of patients with Barrett's metaplasia or adenocarcinoma in a population with symptoms of gastroesophageal reflux disease.

Prior studies using immunoblotting assay reported changes in Carcinoembryonic Antigen (CEA) subcellular distribution and expression between normal and malignant tissues. Other authors observed that elevated serum CEA levels were considered useful in early detection of relapse in patients with resectable adenocarcinoma of

## Biomarkers in esophageal adenocarcinoma

esophagus (36). In a previous report, the authors found not significantly different concentrations of biomarkers between carcinoma and normal esophageal mucosa (37). However, it has been shown high tumor cytosol CEA levels are associated with best clinical outcomes in patients with lung cancer (38).

### 8. PREDICTIVE BIOMARKERS

Biomarkers may also serve as predictive tests for targeted treatments for cancer or for premalignant conditions.

Cyclooxygenase (COX)-2 plays a central role in the production of prostaglandins and is a mediator of angiogenesis and tumor growth. It is inducible through the action of cytokines and endotoxins which normally block cellular death pathways.

Cancer cells might avoid apoptosis by increased synthesis of COX-2. Downstream proangiogenic actions of these products include: production of VEGF; enhanced endothelial cell survival via Bcl-2 expression and Akt signaling; induction of MMPs; activation of EGFR-mediated angiogenesis; and suppression of IL-12 production (39-42).

Patients with adenocarcinoma of the esophagus with high COX-2 expression are more likely to develop distant metastases and local recurrences, and survival was independently associated with reduced survival (40).

This effect was not so pronounced in adenocarcinomas that originated from the cardia (41).

Selective inhibition of COX-2 activity suppresses angiogenesis and induces apoptosis. Therefore, COX-2 inhibitors can possibly be used for the treatment of adenocarcinoma of the esophagus and GEJ (42).

COX-2 expression has been reported in both colorectal adenomas and adenocarcinomas and in Barrett's metaplasia, dysplastic mucosa, and esophageal adenocarcinoma. COX-2 expression has been reported as an independent adverse prognostic indicator in esophageal adenocarcinoma (43), and an argument can therefore be made for trials that test the efficacy of COX-2 inhibitors as adjuvant-targeted treatments.

### 9. CONCLUSION

Research in oncology is ever evolving and new concepts regarding dissemination are constantly developed. Many of these promising concepts have not (yet) been tested on adenocarcinoma of the

esophagus and GEJ and their prognostic value is thus still unknown. One attractive concept is about the role of stem cells. Stem cells have the function to maintain the integrity of tissues such as the intestinal epithelium and have the ability to perpetuate themselves through self-

renewal and to generate mature cells of a particular tissue through differentiation.

Despite advances in multimodality therapy, the prognosis for esophageal adenocarcinoma remains poor. It therefore seems likely that progress with this malignancy will only be made with early detection, prevention, and a clearer understanding of its etiology and tumor biology. Although several molecular alterations have been described in Barrett esophagus, and have been suggested as clinically useful biomarkers to predict progression to invasive esophageal adenocarcinoma, it is anticipated that relatively few will ultimately prove to be of value. To date, only a limited number of phase 3 and 4 studies (as defined by the NCI-EDRN) of biomarkers associated with Barrett esophagus have been completed.

Many studies have evaluated a range of putative tissue biomarkers that might assist in the stratification of the risk of progression of Barrett's metaplasia to adenocarcinoma. Currently only DNA content as measured by flow cytometry and 17p allelic imbalance represent biomarkers that are prospectively predictive of progression, but such results require verification in large multicenter trials. There have been conflicting reports about the efficacy of p53 immunostaining, and it is clear that p53 mutation is not always accompanied by protein overexpression.

No biomarker has yet emerged that is superior to histological identification of dysplasia. The wide variation in the reported rates of progression of dysplasia to malignancy and the problems associated with the reproducibility of such a diagnosis are strong arguments to continue the search. It remains to be seen whether high-throughput hypermethylation analyses (44) or transcriptional profiling (45) can prospectively identify molecularly distinct but histologically indistinguishable high-risk groups of BM patients. Fewer studies have evaluated blood or urine biomarkers, but such approaches could play an even more important role in the early detection of esophageal adenocarcinoma. Biomarker discovery programs are increasingly inextricably linked to the search for novel targeted treatments and to chemoprevention.

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