

Molecular biology of Barrett's esophagus and esophageal cancer: role of p⁵³

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Despite recent advances in multimodality therapy, the prognosis for invasive esophageal cancer is poor, with a five-year survival generally below 10%. While improvements in survival may be anticipated with early detection, improved staging, and rational use of adjuvant therapies, it is likely that significant progress in the treatment of this disease will only occur with an improved understanding of its tumor biology, and careful evaluation of clinically relevant molecular markers.

Over the past decade, various molecular alterations have been reported in human esophageal cancers. As the p53 tumor suppressor gene appears to have a central role in human neoplasia, the aim of this review is to discuss the potential clinical applications of this biomarker in esophageal cancer.

ESOPHAGEAL CANCER EPIDEMIOLOGY^[1]

Squamous cell carcinoma of the esophagus is one of the most frequent malignancies worldwide. The epidemiology of this disease is characterized by a striking geographic variation in incidence, not only between countries, but also within distinct geographic regions and among ethnic groups. Epidemiologic and experimental studies from high-incidence areas have implicated several environmental factors with the development of esophageal cancer, including nutritional deficiencies, dietary nitrosamine precursors, alcohol intake and tobacco smoking. However, the relative influence of each factor appears unique to the region studied.

Recent reports from North America and Europe confirmed clinical suspicions that adenocarcinomas of the lower esophagus and cardia were being seen more frequently. The 10% annual rate of increase

in white males exceeded that for any other solid tumors. Furthermore, a proximal shift in gastric carcinomas towards the upper third has also been described in recent years. Although the factors for this changing pattern of disease are unknown, molecular epidemiologic studies may provide further insight into the etiology and biology of esophagogastric carcinomas.

ESOPHAGEAL ADENOCARCINOMA

Primary esophageal adenocarcinomas are frequently confused with proximal gastric (or cardia) cancers. Despite apparently different clinical and biologic behaviour, there currently appears to be no clear way to accurately distinguish between these tumors. This is of particular importance in view of the changing epidemiology of this disease, and in planning treatment strategies. One recent classification^[2] proposed a definition based on tumor measurements related to the anatomic esophagogastric junction (EGJ). Primary esophageal adenocarcinomas were defined as tumors centred 1 to 5cm above the EGJ; cardiac carcinomas, between 1cm above and 2cm below the EGJ; and subcardial gastric carcinomas, with a tumor centre from 2cm to 5cm below the EGJ.

In 1991, we proposed guidelines in an attempt to establish the primary esophageal origin of adenocarcinomas^[3]. These criteria (summarized below), incorporating clinical and pathologic characteristics of these tumors, were determined by preoperative endoscopy, radiology, at surgery, and on pathologic examination of the resected foregut.

a. An associated Barrett's epithelium. When present, this is virtually a diagnosis of a primary esophageal adenocarcinoma. However, approximately 50% of tumors will not have a demonstrable Barrett's mucosa, presumably because this has been incorporated into the tumor mass. In this situation, the following criteria assume increasing importance.

b. Greater than 75% of the tumor mass involving the tubular body of the esophagus.

c. Direct histologic invasion of periesophageal tissues.

d. Minimal gastric involvement.

e. Clinical symptoms of esophageal obstruction (i.e. dysphagia).

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These criteria have been crucial to the design and conduct of our laboratory/translational studies, and are increasingly used in current clinical practice.

BARRETT'S ESOPHAGUS^[4]

Barrett's esophagus is characterized by replacement of normal squamous epithelium, by intestinalized columnar epithelium. Previous definitions of a columnar epithelium lined esophagus required variable lengths of replacement proximal to an arbitrary 2cm-3cm of "normal columnar lining" of the lower esophagus. However, the histologic finding of intestinal type goblet cells is now accepted to be a prerequisite for the diagnosis of Barrett's mucosa, which incorporates "short segment" Barrett's esophagus (i.e. less than 3cm).

Barrett's esophagus is thought to be an acquired condition resulting from chronic gastroesophageal reflux disease. In symptomatic patients (i.e. dyspepsia, heartburn), the prevalence of Barrett's epithelium is estimated at 10%, whereas less than 1% of asymptomatic patients will have this diagnosis. The importance of this finding is that it is premalignant. The risk that patients with preexisting Barrett's mucosa will develop invasive esophageal carcinoma was estimated by two prospective studies to be at least fifty times greater than the general population. Dysplasia is widely regarded as the precursor of invasive cancer, and high-grade dysplasia in Barrett's epithelium is frequently associated with primary esophageal adenocarcinoma. Dysplastic change may be characterized histologically by experienced pathologists in biopsy specimens obtained at esophagoscopy. Recent reports suggest that endoscopic surveillance can detect early adenocarcinoma in Barrett's epithelium, and that early detection and surgical resection may decrease the mortality rate for esophageal carcinoma^[5].

THE p53 TUMOR SUPPRESSOR GENE^[6,7]

Certain genes appear necessary to regulate cell growth and prevent oncogene-driven clonal expansion and uncontrolled cellular proliferation. Loss of tumor suppressor function (thereby providing a selective growth advantage for clonal expansion) is thought to require inactivation of both alleles, and this may occur through several mechanisms including chromosomal deletion, point mutation, or by neutralization of the protein product by interaction with other cellular proteins.

The p53 protein was first discovered in 1979 as a cellular 53-kD nuclear phosphoprotein bound to the large transforming antigen (T antigen) of the Simian virus 40 (SV40), and subsequently to oncoproteins from other tumor viruses. The normal

function of p53 protein is not known with certainty, but it is believed to be a transcription factor which plays a role in cell cycle progression. DNA damage induces a rapid accumulation of cell nuclear p53 protein, resulting in transactivation of target genes including the cyclin kinase inhibitor p21waf-1, bax-1, a regulator of apoptosis, and other key proteins regulating DNA replication, transcription and repair.

The p53 gene is localized to the short arm of chromosome 17 (17p13), and comprises 11 exons with five evolutionarily conserved domains within the coding region. 95% of known p53 mutations fall within these DNA-binding regions, the majority of which are missense, which lead to stabilization and cell nuclear accumulation of p53 protein. Various p53 mutations may result in different biological properties, and this varies depending on the cell system. The molecular basis of p53 mutations / function is not fully understood. Exons encoding the N and C terminal domains contain a higher proportion of nonsense mutations.

The diversity of p53 mutations in human solid tumors suggests that this may be a potentially useful biomarker for molecular epidemiologic studies. For example, mutations arising from endogenous events, such as methylation and deamination of cytosine to thymine, result in C to T transitions at CpG dinucleotides. Mutations do not form at an equal rate at all base positions, and a strand bias in distribution suggests exogenous mechanisms. Mutation patterns may also suggest DNA damage induced by exogenous carcinogens. For example, A:T to T:A transversions in patients with hepatic angiosarcomas following exposure to vinyl chloride; and G to T transversions in patients with lung cancers associated with tobacco (benzo-a-pyrene) exposure. The most frequently mutated p53 codons in lung cancer are 157, 248, and 273, which may be preferential sites for DNA adduct formation. Codon 157 is rarely mutated in other tumors, and may be seen as a "hot spot" for lung carcinoma. By contrast, while codons 248 and 273 are frequently mutated in several other cancers, patterns of mutation may differ at these sites. For example, in breast and colorectal cancers, C to T transitions at CpG dinucleotides suggest an endogenous mechanism.

p53 ALTERATIONS IN ESOPHAGEAL CANCER AND BARRETT'S MUCOSA

p53 gene mutations were first reported in esophageal squamous cell carcinomas by Hollstein *et al* in 1990^[8]. Using strict criteria to define adenocarcinomas of esophageal (vs. gastric) origin, we reported p53 mutations in primary esophageal adenocarcinomas and associated Barrett's

epithelium^[3]

These original observations have now been confirmed by other investigators, documenting additional p53 mutations, altered expression of p53 mRNA, p53 protein accumulation and loss of heterozygosity of chromosome 17p^[9].

Patterns of p53 mutations differ widely for squamous cell carcinomas and adenocarcinomas of the esophagus. To date, most esophageal tumors studied have been squamous cell carcinomas, where 46% of tumors were found to have p53 mutations, predominantly at codons 175, 193, 194, 195 and 270. The high frequency of G:C to A:T transversions and A to T transitions at CpG dinucleotides, suggesting that p53 mutations in these tumors may occur spontaneously by deamination or mismatch repair.

POTENTIAL CLINICAL APPLICATIONS^[10]

It has been proposed that p53 alterations in esophageal tissues may have potential clinical applications: for early diagnosis of invasive esophageal cancer in patients with Barrett's esophagus undergoing endoscopic surveillance, for staging, as a stratification factor in future clinical trials, and as a molecular target for novel anticancer therapies.

Early detection

Although the natural history of Barrett's epithelium is not known, it is considered a premalignant tissue. Endoscopic surveillance is currently used to identify patients at increased risk for developing malignancy. However, conventional histologic markers such as dysplasia do not reliably predict the development of invasive carcinoma.

The timing of p53 alterations in esophageal tumorigenesis is not known with certainty. However, the finding of p53 mutations in metaplastic (non dysplastic) Barrett's mucosa, suggests this may be an early event in esophageal tumorigenesis. This is in keeping with other tumors of the upper aerodigestive tract (i.e. oropharynx, lung), where p53 mutations are believed to occur as relatively early molecular events. The finding of a p53 alteration in Barrett's mucosa, may, in the future, be used to identify subsets of patients at increased risk for esophageal malignancy, prompting further intervention (increased surveillance or resection). Prospective clinical studies are warranted to test this hypothesis.

Prognosis

Fundamental differences in esophageal tumor biology may explain different clinical outcomes for patients with histologically similar tumors within each well defined stage. Incorporation of molecular

markers, such as p53, into conventional clinicopathologic staging schema may improve reliability of predicting tumor recurrence, metastasis or survival.

To date, published studies evaluating the prognostic value of p53 protein in esophageal cancer have reported conflicting results. We recently reported a prospective study, evaluating p53 mutations and protein accumulation in esophageal adenocarcinomas, in an attempt to clarify the role of p53 as a predictor of survival following surgical resection. We found that p53 alterations were associated with significantly reduced disease-free and overall survival, by both univariate and multivariate analysis, suggesting p53 to be a potentially useful prognostic marker in this disease^[11].

Therapy

There has been increased interest in the use of chemotherapy in the multimodality therapy of esophageal cancer. It has been suggested that p53 may be used to stratify patients in future clinical trials evaluating induction chemotherapy. This approach was recently supported by experimental studies of esophageal cancer cell lines, where mutational status of p53 protein was predictive of chemosensitivity^[12].

The correction of underlying molecular alterations for preventing or treating cancer is a goal of gene therapy. Current approaches use modified or attenuated viral vectors to deliver genetic material into the tumor cell nucleus. Preliminary results with lung cancer have been encouraging, and it is believed that this approach will be successful for other upper aerodigestive tract tumors.

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