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p53 and the Malignant Progression of Barrett's Esophagus

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Barrett's esophagus (BE) is a metaplastic disorder in which specialized columnar epithelium replaces healthy squamous epithelium (intestinal metaplasia). Even though its pathophysiology and the steps of its neoplastic progression are not completely understood, BE can be considered as a complication of gastroesophageal reflux disease (GERD). Given that esophageal adenocarcinoma, which is continually increasing in the Western world, still has a poor prognosis and suffers from late diagnosis, and because BE is a precancerous lesion, there is a strong need for good molecular markers of malignant progression in Barrett's metaplasia (BM). The aim of this review is to examine the published data regarding the role that assessment of p53 may play in the management of BE, trying to understand if it may be a useful marker to early diagnose BE malignant transformation. J. Cell. Physiol. 206: 574–577, 2006. © 2005 Wiley-Liss, Inc.

Barrett's esophagus (BE) is a condition in which the normal multi-layered squamous epithelium is replaced by a metaplastic columnar one of any length, predisposing the esophagus to the development of adenocarcinoma. Instead of pathologists' conception, according to gastroenterological guidelines and recent reports, we can talk about BE only in case of intestinal metaplasia (IM), and not of other columnar types of epithelium found in the lower third of esophagus.

During an upper endoscopy, BE can just be suspected as its certain diagnosis is based on a histologic evaluation of endoscopic biopsies.

Although its pathophysiology is not completely understood, Barrett's metaplasia (BM) can be considered as a complication of gastroesophageal reflux disease (GERD); in other words, when defense mechanisms in the esophageal mucosa are chronically overwhelmed by harmful agents, it develops as a healing process protecting the esophagus from further damage (Guillem, 2005). The severity of GERD correlates with the length of metaplastic esophageal mucosa and further changes occurring in it. For example, if the length of BE is < 3 cm, it is defined short-segment BE (SSBE), which has a minor risk of malignant progression and correlates with a less severe form of GERD than that of patients with long-segment BE (LSBE) (length > 3 cm) (Csendes et al., 2002; Wakelin et al., 2003; Spechler, 2004).

The pathophysiological sequence whereby GERD leads to adenocarcinoma (GERD $\rightarrow$ inflammation $\rightarrow$ IM $\rightarrow$ dysplasia $\rightarrow$ adenocarcinoma) (Buttar and Wang, 2004) is based on different possible mechanisms that are not mutually exclusive and might even be reversible, according to different molecular patterns turning protein transcription on and off (Riddell, 2005).

If the mucosal alteration is a very short segment in the region of the gastro-esophageal junction (GEJ), as observed in 5–34% of patients undergoing upper endoscopy, histology cannot distinguish between SSBE or IM of the gastric cardia. The two lesions have a different pathogenesis (dealing the former with GERD and the latter with H. Pylori, which instead seems to be protective against BE) (Goldblum et al., 2002; Abe et al., 2004; Sharma et al., 2004) and two different risk of malignancy (higher for SSBE: at most 0.5% per year) (Sharma et al., 2000, 2004; Spechler, 2004).

Possible management strategies for BE with high-grade dysplasia (HGD), representing the highest risk of cancer, are: endoscopic ablative therapies or endoscopic mucosal resection (both if submucosa and mucosal lymphatic system have not been invaded), esophagectomy, intensive endoscopic surveillance (especially for elderly patients who sometimes, however, develop adenocarcinoma) associated to acid suppression therapy (AST). Literature does not offer enough data to decide what are the most appropriate therapies for BE with dysplasia, as the follow-up duration, in most studies on dysplasia treatments, is substantially less than 5 years. Opinions about endoscopic therapies are discordant, but recent studies suggest that they usually leave metaplastic or neoplastic epithelium with malignant potential behind (Spechler, 2005). Moreover studying the effect of photodynamic therapy (PDT) at the genetic level, it has been discovered that despite of endoscopic removal of BE, histologically complete elimination cannot be achieved in all cases, and however, molecular and genetic abnormalities persist, not preventing the malignant potential of the lesion (Krishnadath et al., 2000; Wolfsen et al., 2004; Hage et al., 2005). Also AST, may be effective in preventing further DNA damage, may not alter neoplastic progression in BM if key genes involved in DNA repair and cell cycle control, particularly p53, are already defective (Carlson et al., 2002).

Surveillance for detection of dysplasia is actually the gold standard to select patients with higher risk of malignant progression; but as histopathologic evidence of dysplasia is a subjective method of diagnosis (Skacel et al., 2002; Younes et al., 2003), and as its natural history is incompletely defined, better markers for detecting patients at high risk for adenocarcinoma are needed. This needing is supported by the recognition of BE as a premalignant lesion, and by real rise in incidence of esophageal adenocarcinoma (a sixfold

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increase in the USA between 1975 and 2001, with a mortality that has increased more than sevenfold) (Pohl and Welch, 2005).

We know that the *p53* gene plays an important role in the regulation of apoptosis and cell growth, so that the loss of wild-type activity is associated with uncontrolled cell cycle progression and tumor formation (Halm et al., 2000; Woodward et al., 2000). The aim of this review is to assess the possible role of p53 as a marker to early diagnose malignant potential in BE.

WHAT IS p53?

p53 is a tumor suppressor involved in controlling cell proliferation and able to inhibit the transformation of cells in culture by various oncogenes. In fact, a large increase of this nuclear phosphoprotein is found in many transformed cells or lines derived from tumors, as the loss of these controls provides a growth advantage to them; instead, all normal cells have low levels of p53 and can grow in an unrestrained manner that is usually inhibited by p53.

p53 mutants are dominant negatives, as they overwhelm the wild-type protein and prevent it from functioning; they also have a role in the characteristic instability of the cancer cell genome (Lewin, 2000; Gan et al., 2003).

p53 functions

Two types of events can be triggered by the activation of p53: growth arrest and apoptosis. The outcome can follow various pathways, involving many different molecules (Fig. 1), and depends on which stage of the cell cycle has been reached; however, some cell types are more prone to show an apoptotic response than others.

In cells early in G1, p53 triggers a checkpoint blocking further progression through the cell cycle; this allows the damaged DNA to be repaired before the cell tries to enter S phase. But if a cell is committed to division, then p53 triggers a program of cell death.

This suggests that apoptosis plays an important role in inhibiting tumorigenesis probably because it eliminates potentially tumorigenic cells, and that the failure of p53 to respond to DNA damage is likely to increase susceptibility to mutational changes that are oncogenic (Lewin, 2000; Gan et al., 2003).

We also have to remember that the higher levels of both proliferation and apoptosis mean an increased cell turnover in Barrett's epithelium; apoptosis seems to maintain tissue homeostasis, which is gradually lost in the metaplasia-dysplasia-carcinoma-sequence of BE as it is regulated by p53 (Halm et al., 2000). Proteins that activate p53 behave as tumor suppressors; proteins that inactivate p53 behave as oncogenes.

p53 and BE

Dysplasia is associated with an increased risk of malignant transformation in BE (10–30% of HGD develop cancer within 5 years of the initial diagnosis) (Younes et al., 2003), but the rate of progression varies among studies, also because of pathologists' interobserver variability (Skacel et al., 2002; Younes et al., 2003) and biopsy sampling errors. However, progression through dysplasia to malignancy develops as a multistep process involving genomic instability and the presence of aneuploid cell populations. In BM, as a consequence of oxidative DNA damage due to gastroesophageal reflux, there is an increased percentage of cells in the G0/G1 or G2/M phases of the cell cycle to enable DNA repair; this is sometimes sequentially followed by p53 gene mutation and protein accumulation, DNA aneuploidy, HGD, and carcinoma (Younes et al., 2000). So the matter is to assess what is the best predictor of neoplastic risk in BE between histopathological diagnosis of dysplasia and p53 detection.

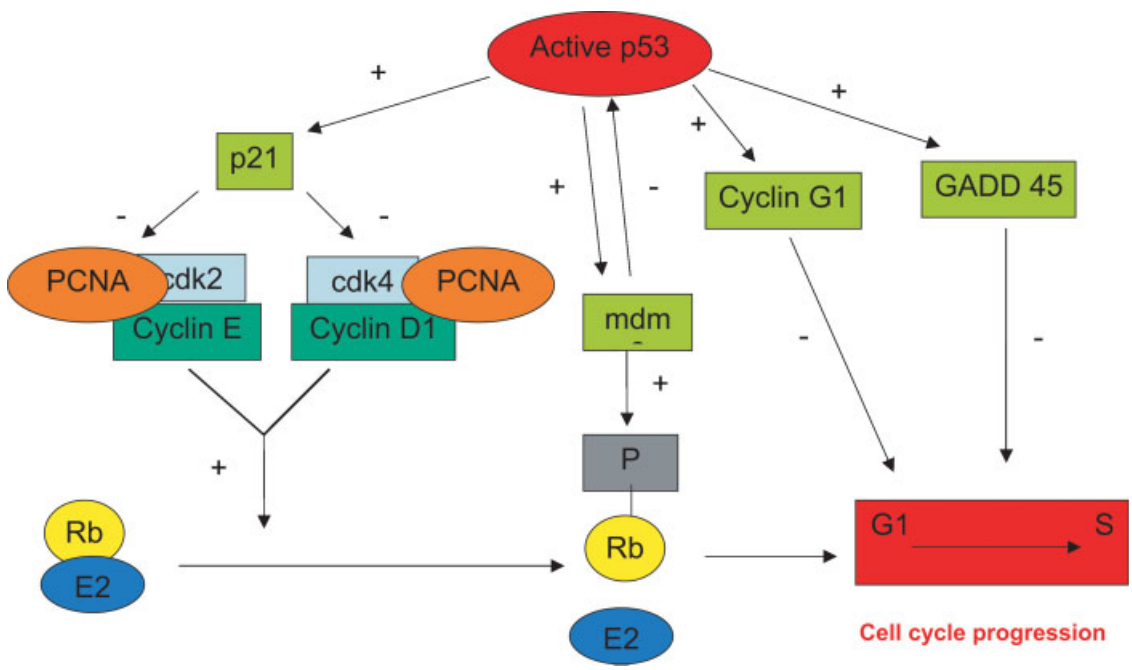

Fig. 1. The role of p53 in cell cycle control. Transition from the G1 phase of the cell cycle into the DNA synthesis S phase is guarded by p53. In case of DNA damage, the Mdm2 protein is inhibited to interact, and p53 will accumulate in the nuclei. p53 indirectly can inhibit phosphorylation of the *RB* gene and prevent cells from progressing through the restriction point late in the G1 phase of the cell cycle and enter S phase.

Trying to use p53 as a marker of malignant progression, we have to answer three questions:

1. What is the best method for this purpose?
2. In what phase of the multistep process from metaplasia to carcinoma, p53 mutations occur and are detectable?
3. Does p53 play the same role in malignant progression of IM of the gastric cardia?

Let's try to answer the first question. As p53 accumulation detected by immunohistochemistry (IHC) has a sensitivity of 88% and a specificity of 75% for progression of LGD to HGD/cancer; using this method in conjunction with histological diagnosis of LGD, patients with LGD and p53-positive biopsies (being more likely to develop HGD/cancer) should be followed up more closely than those with LGD and p53-negative biopsies (Ohbu et al., 2001; Weston et al., 2001; Skacel et al., 2002). However, according to literature, about 20% of p53 gene alterations are complex mutations with no p53 protein synthesis, and IHC would miss them. Moreover, mutational analysis of p53 by molecular biology techniques and p53 accumulation by IHC have been proved to be mostly concordant in adenocarcinoma and HGD but frequently discordant in LGD (Bian et al., 2001). This suggests that mutational analysis of p53 should be considered a more reliable method.

The second question is more difficult to answer. In fact, mutations of p53 have always been thought to accumulate more in highly dysplastic epithelium than in non-dysplastic epithelium (Schneider et al., 2000; Kimura et al., 2001; Reid et al., 2001; Dolan et al., 2003). Instead of most reports dealing only with HGD and cancer, it has been recently suggested that genetic abnormalities of this molecule may exert their influence earlier in BE malignant progression (Barrett et al., 2003; Fahmy et al., 2004); recent studies even assess that loss of heterozygosity (LOH) on 17p is a frequent event in BE also in the absence of dysplasia and adenocarcinoma, thus raising the chance of using the presence of this abnormality as a marker for risk stratification within endoscopic surveillance programs (Dunn et al., 2000; Sanz-Ortega et al., 2003; Suspiro et al., 2003).

At last, for the third question, in spite of the different aetiologies of SSBO and IM of the gastric cardia, the cell-cycle response is similar and both of the lesions may have malignant potential (Trudgill et al., 2003; Segal et al., 2004); so we can apply the considerations so far performed to these conditions.

In conclusion, histodiagnosis of dysplasia remains the best predictor of neoplastic progression in BE. However, as p53 surely plays an important role in tumorigenesis, and as IHC has been proved to fail in some cases, next studies have to establish the best technique able to detect p53 mutations so early to restrict endoscopic follow-up to patients at real risk of malignancy.

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