Re: Clonal Expansion and Loss of Heterozygosity at Chromosomes 9p and 17p in Premalignant Esophageal (Barrett’s) Tissue

The Journal recently published an article by Galipeau et al. (1) about loss of heterozygosity (LOH) at chromosomes 9p and 17p and clonal heterogeneity in Barrett’s esophagus. The article described a high prevalence of LOH at 9p and 17p in endoscopic biopsy specimens and found that LOH at 9p was more common than LOH at 17p in diploid samples. The biopsy samples contained a mosaic of patterns of LOH and ploidy, indicating that a high degree of clonal heterogeneity exists in Barrett’s esophagus.

One important issue was not addressed by the article by Galipeau et al., however, because the samples that were investigated were not clearly histopathologically classified. Dysplasia often arises in the intestinal metaplasia of Barrett’s esophagus as multiple foci. Moreover, when high-grade dysplasia is present, it is often mixed with areas of low-grade dysplasia and intestinal metaplasia without dysplasia. However, these precursor lesions are not endoscopically distinguishable within the segment of Barrett’s esophagus. In addition, one cannot exclude the possibility that several patients may have already developed a microinvasive carcinoma, since it is found in 45%–75% of resection specimens of patients undergoing esophagectomy for high-grade dysplasia (2–4). Since histopathologic evaluation was not performed on every sample actually investigated by Galipeau et al. (1), the endoscopic specimens could presumably have presented the whole spectrum of the metaplasia–dysplasia–adenocarcinoma sequence. Thus, the clonal heterogeneity observed as a mosaic of clones and subclones with different patterns of LOH is not surprising, and it is possibly a reflection of the inclusion of several types of lesions, including intestinal metaplasia, low- and high-grade dysplasia, or even carcinoma.

We recently published a study detailing with genetic alterations in Barrett’s esophagus (5). Applying exclusively morphologically linked techniques, such as microdissection and comparative genomic hybridization or *in situ* hybridization, we were able to correlate molecular genetic findings to specific steps of the metaplasia–dysplasia–adenocarcinoma sequence in Barrett’s esophagus. Of interest, we found genetic loss at chromosome 9p to be more prevalent than DNA loss at 17p in premalignant Barrett’s esophagus, and both become steadily more frequent along the metaplasia–dysplasia–adenocarcinoma sequence (5). Moreover, a high degree of heterogeneity of c-erbB2 gene amplification and overexpression was clearly evident in high-grade dysplasia and adenocarcinoma by fluorescence *in situ* hybridization and immunohistochemistry (5–7).

Since the histopathology of the specific steps of the metaplasia–dysplasia–adenocarcinoma sequence has a fundamental impact on our understanding of the development of Barrett’s adenocarcinoma, the scientific value of molecular genetic data is especially high when it is obtained as a result of the direct correlation between molecular genetic and histopathologic findings in the same biopsy specimen. Thus, although the flow cytometric method used by Galipeau et al. (1) has its advantages and the findings of the study are potentially important, an accurate histopathologic evaluation of each sample analyzed would greatly add to the value of the findings in terms of the understanding of the molecular biology of the metaplasia–dysplasia–adenocarcinoma sequence in Barrett’s esophagus.

Axel Walch

James Mueller

Heinz Höfler

Martin Werner

References


Notes

Affiliations of authors: A. Welch, H. Höfler, Technical University Munich, Institute of Pathology, Munich, Germany, and GSF-National Research Center for Environment and Health, Institute of Pathology, Neuherberg, Germany; J. Mueller, Technical University Munich, Department of Surgery, Munich; M. Werner, Technical University Munich, Institute of Pathology, Munich.

Correspondence to: Axel Walch, M.D., GSF-Forschungszentrum für Umwelt und Gesundheit, Institut für Pathologie, Ingolstädter Landstraße 1, D-85764 Oberschleißheim, Germany (e-mail: Walch@gsf.de).

Response

We appreciate the comments of Walch et al. concerning the differences between our respective studies. Both groups used objective methods (comparative genomic hybridization [CGH] versus fluorescent loss of heterozygosity analysis) to assess somatic genetic abnormalities in Barrett’s esophagus. Both groups provided similar data that progression is associated with nonlinear evolution of neoplastic cell lineages leading to clonal heterogeneity in Barrett’s epithelium.

Walch et al. applied “exclusively morphologically linked techniques” to correlate their findings to specific histologic grades. However, routine histologic interpretation of dysplasia in Barrett’s esophagus is subjective, and numerous studies have found that the results are not generally reproducible. For example, in our study of observer variation, there was only 48% agreement on all grades of a dysplasia classification system, and another study reported 63% agreement (1,2). Objective, reproducible methods are essential for independent
validation of results by other investigators. We and others (3,4) have prospectively validated objective flow cytometric biomarkers that have high interlaboratory reproducibility. Furthermore, flow sorting purifies neoplastic clones that can arise independent of morphologic changes (5). It is unlikely that clonal heterogeneity can be attributed simply to histologic heterogeneity because extensive clonal heterogeneity has been documented independent of histologic findings (5), which is consistent with the observations of Walch et al. that the same histologic grade can have multiple, different chromosomal abnormalities in different patients.

Other reasons exist to re-evaluate assumptions underlying the proposed linear model for disease progression in Barrett’s esophagus from metaplasia to low-grade dysplasia to high-grade dysplasia to adenocarcinoma. Clinical studies (3,6) have found that these intermediate events can regress, progress, or remain stable. This variable behavior may be due to diagnostic inconsistency in histologic interpretation, exposures to risk and protective factors, or biological properties of the clones themselves. We have observed some clones that appear to progress slowly or not at all, whereas others progress (5). Walch et al. described copy number changes in lower grade histologic lesions that were not present in higher grade lesions from the same patient, which is not predicted by a linear model. However, DNA copy number changes detected by CGH or other techniques can be caused by a number of mechanisms and, in isolation, may not be stable clonal markers that can be used to follow the evolution of neoplastic cell lineages. Nevertheless, our two respective studies and other studies indicate that genetic instability leads to clonal heterogeneity that can arise early and that clonal evolution drives neoplastic progression (3,5).

Thus, rigid adherence to an assumed linear histologic pathway may limit interpretation of results.

Although the surgical literature contains numerous reports of patients found to have cancer in surgical resections after an endoscopic diagnosis of high-grade dysplasia, the weaknesses of this literature have been well described (6,7). None of these studies used the systematic, intensive biopsy protocols repeated at closely timed intervals recommended for the surveillance of high-grade dysplasia, which consistently detect cancers when they are small and early (7). In our study, a mean of 2.7 endoscopies (range, 1–16) and 78.4 histologic biopsy specimens (range, 9–490) were evaluated in the 61 patients without detection of cancer, making it highly unlikely that undetected cancers contributed substantially to our results (7).

References


Note

Correspondence to: Brian J. Reid, M.D., Ph.D., Divisions of Human Biology and Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109 (e-mail: bjr@fhcrc.org).