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Immunohistochemistry as a surrogate for molecular subtyping of gastric adenocarcinoma☆,☆☆

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Summary The Cancer Genome Atlas Research Network recently classified gastric adenocarcinoma into 4 molecular subtypes: Epstein-Barr virus–positive tumors, microsatellite-unstable tumors, tumors with chromosomal instability, and genomically stable tumors. We theorized that immunohistochemistry might be useful in similar categorization and that that HER2 expression might relate to subtype. We stained 104 gastric adenocarcinomas for MLH1, p53, and EBER in situ hybridization. We grouped them based on staining pattern and compared the groups. Cases were categorized as follows: group 1 (EBER positive), 7 cases (7%); group 2 (MLH1 deficient), 17 cases (16%); group 3 (aberrant p53 staining, EBER negative, retained MLH1), 40 cases (38%); group 4 (unremarkable staining), 40 cases (38%). This distribution was comparable to that found by the Research Network after accounting for the *TP53* mutation rate in the chromosomal instability group. Group 1 patients had significantly longer follow-up times (median, 70 months versus 13 months for other groups; $P = .0324$). No group 2 cases overexpressed HER2. In group 3, 3 of 40 cases were HER2 immunohistochemistry positive, but 7 of 27 were HER2 positive by fluorescence in situ hybridization. Staining offers an efficient, reasonably accurate alternative for molecular subtyping of gastric adenocarcinoma, although some cases with chromosomal instability cannot be identified. These findings have potential prognostic and therapeutic implications.
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1. Introduction

Several classification schemes exist for gastric adenocarcinoma [1]. One of the most enduring, the Laurén classification, divides tumors into intestinal type and diffuse type based primarily on microscopic appearance [2]. The World Health Organization expands on morphologic subtypes, including varieties such as papillary, tubular, and mucinous adenocarcinoma [3]. Other classifications group tumors based on clinical stage (early versus advanced disease) and gross configuration (polypoid, fungating, ulcerated, and infiltrative) [3].

The Research Network of The Cancer Genome Atlas (TCGA) recently categorized gastric adenocarcinoma into 4

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subtypes via molecular analysis: Epstein-Barr virus (EBV)–positive tumors, microsatellite-unstable tumors with *MLH1* hypermethylation, genomically stable tumors, and tumors with chromosomal instability (CIN), of which 71% harbor *TP53* mutation [4]. Although similar molecular alterations have been described in isolation, TCGA proposed a streamlined, stepwise process for characterizing a gastric carcinoma based on its predominant molecular profile, which may have clinical relevance based on subtype prognosis and targeted therapy options.

Given that alterations in *MLH1* and *TP53* and infection with EBV were prominent findings in TCGA's classification scheme, we hypothesized that immunohistochemical (IHC) staining and in situ hybridization of tissue samples from gastric adenocarcinomas could approximate the scheme in a simple and cost-effective manner. Furthermore, given that treatment of gastric carcinoma is influenced by amplification of the *ERBB2* gene (which encodes the HER2 protein) [5], we set out to determine the relationship, if any, between HER2 overexpression and TCGA's defined molecular subtypes.

2. Materials and methods

With appropriate Research Subjects Review Board approval, we identified 104 cases of gastric adenocarcinoma in our departmental archives with available clinicopathological patient data, hematoxylin and eosin–stained slides, and tissue blocks. This included HER2 data (evaluated by IHC and/or fluorescence in situ hybridization [FISH]). We performed IHC staining for MLH1 (clone G168-15, dilution 1:50; Biocare Medical, Concord CA) and p53 (clone DO-7, ready to use; Dako North America, Carpinteria, CA), and EBER in situ hybridization (Biocare Medical) on sections from all cases. Both MLH1 and p53 were detected using high-pH (pH 9) heat-induced epitope retrieval and the Dako Omnis Flex HRP kit with 3,3′-diaminobenzidine. The EBER probe was detected using the RISH HRP Detection kit from Biocare Medical.

The slides were interpreted as follows: complete loss of MLH1 staining, with appropriate retention in background nonmalignant tissue, was interpreted as a positive result [6]; strong p53 nuclear expression in at least 70% of tumor nuclei was interpreted as a positive result [7]; and identifiable nuclear staining for EBER was interpreted as a positive result [8].

We then stratified all of the cases into 4 groups based on the staining results using the same algorithm as TCGA (Fig. 1): EBER-positive cases were placed into group 1 (corresponding to TCGA's EBV-positive group) (Fig. 2A); of the remaining cases, MLH1-deficient cases were placed into group 2 (TCGA's microsatellite-unstable group) (Fig. 2B); of the remaining cases, p53-aberrant cases were placed into group 3 (TCGA's CIN group) (Fig. 2C); and the remaining cases were placed into group 4 (TCGA's genomically stable group).

We compared the 4 groups by patient age, sex, clinical follow-up, tumor location (gastroesophageal junction [GEJ]–cardia, fundus-body, or antrum-pylorus), morphologic tumor pattern (intestinal type or diffuse type), American Joint Commission on Cancer stage [9], and available HER2 data using χ^2 and Wilcoxon rank-sum tests as appropriate to the data. For biopsy specimens, clinical and radiographic data were used to determine stage when available. A Cox proportional hazards model was used to evaluate hazard of death from disease. All analyses were carried out using SAS9.4 software (SAS Institute, Cary, NC) on a Windows 7 platform.

3. Results

The 104 cases, each from a different patient, included 46 biopsies and 58 resections. Staining results categorized the cases as follows: group 1 (EBER positive), 7 cases (7%); group 2 (MLH1 deficient), 17 cases (16%); group 3 (strong p53 staining, EBER negative, retained MLH1), 40 cases (38%); and group 4 (unremarkable staining pattern), 40 cases (38%). EBER positivity and MLH1 loss were mutually exclusive; strong p53 staining was seen in 3 EBER-positive and 5 MLH1-deficient cases.

Relevant clinicopathological data are summarized in the Table. Some data were not available for all tumors because of factors such as incomplete/ambiguous clinical histories and lack of resection or clinical staging, typically in patients not amenable to comprehensive treatment.

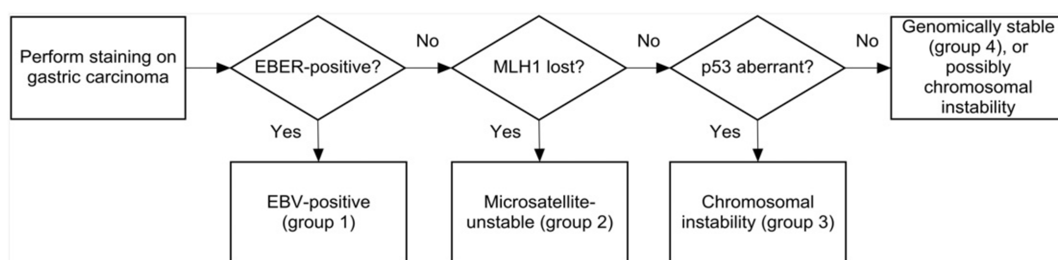

Fig. 1 An algorithm for categorizing gastric adenocarcinoma based on staining pattern. First, all EBER-positive cases are classified as group 1 (EBV positive). Next, all EBER-negative cases that lack staining for MLH1 are classified as group 2 (microsatellite unstable). The remaining cases are classified based on whether they stain strongly for p53 (group 3, chromosomal instability) or not (group 4, genomically stable).

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