Evaluating Mutation Load in Low and High Grade Dysplasia in Barrett’s Esophagus

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RESULTS
Histological evaluation of the presence and degree of dysplasia in patients with Barrett’s esophagus (BE) is often subjective and challenging. We evaluated a panel of molecular markers to analyze the degree of molecular change in conjunction with histologic findings. Biopsy specimens from 20 patients with a diagnosis of Barrett’s (intestinal metaplasia) with dysplasia (n=19), and intestinal metaplasia without dysplasia (n=1) were examined. Multiple microdissection targets (n=57) from formalin-fixed, paraffin-embedded slides were tested for loss of heterozygosity (LOH) mutations and/or microsatellite instability in a panel of 17 microsatellite markers using PCR. The presence/absence of LOH and the proportion of cells affected by LOH were quantitatively determined, with high clonality mutations representing >75% of cells, and low clonality representing 50-75%. We estimated mutation load using values for low and high clonality mutations and microsatellite instability. All targets in this series showed mutations, including those without dysplasia. Microdissection targets with histologic high-grade dysplasia (HGD, range 2.0-6.0, average 3.3) had higher mutation load than low-grade dysplasia (LGD, range 1.0-4.5, average 2.4). Targets with carcinoma in situ (CIS) showed significantly higher mutation load (range 3.0-7.0, average 5.3) than targets showing only high-grade dysplasia. Determining ranges of mutation load around the averages for low/high grade and carcinoma in situ can help characterize neoplastic progression from metaplasia thru dysplasia. Molecular profiling of targets with mutation load determination, may be a useful adjunct to histologic interpretation in determining the presence and degree of dysplasia, and help clarify the potential for biologic aggressiveness in each patient with management/surveillance implications.

CONCLUSIONS
Genomic instability, as represented by mutational load (ML), increases with increasingly severe grade of disease. Cells with the same histological appearance have distinct levels of genomic instability, which may help to explain why some undergo disease progression and others do not. Examining mutation loads (ML) may provide an additional dimension to the observed histology in BE patients, potentially helping to define subsets of patients with greater risk for further disease progression.