CASE REPORT

Liquid biopsy as a novel tool to monitor the carcinogenesis of Barrett's esophagus

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Barrett's esophagus (BE) is associated with an increased risk of developing esophageal adenocarcinoma. For this reason, endoscopic-based surveillance protocols have been developed. This prevention program is, however, burdensome for the patients and expensive for the national health systems. Thus, diagnostic strategies with a low invasiveness and a reduced economic impact are required. This study investigated the power of plasma circulating free DNA (cfDNA) in predicting neoplastic transformation in the natural history of two BE patients who progressed to esophageal adenocarcinoma. Longitudinally collected DNAs from plasma and paired formalin fixed paraffin embedded samples were examined for both loss of heterozygosity (LOH) in areas proximal to *TP53*, *FHIT* and *BRCA2* genes, and mutations in *TP53* gene. Results showed that: (i) early BE molecular alterations are mainly localized proximal to, or within, *TP53* gene; (ii) LOH events present in cfDNA not only retrace the time-matched biopsy profile but better represent the total alterations of the BE epithelium. In conclusion, our findings suggested that LOH analysis in plasma cfDNA could represent an additional, less invasive, diagnostic tool to monitor neoplastic progression of BE epithelium. (Translational Research 2016;176:127–131)

Abbreviations: BE = Barrett's esophagus; EAC = esophageal adenocarcinoma; cfDNA = circulating free DNA; FFPE = formalin fixed paraffin embedded; LOH = loss of heterozygosity; LGD = low-grade dysplasia; HGD = high-grade dysplasia; chr. = chromosome

INTRODUCTION

Barrett's esophagus (BE) is a condition that predisposes to esophageal adenocarcinoma (EAC), an aggressive tumor associated with a dismal clinical outcome. For this reason, individuals with BE are subjected to a stringent program of endoscopic biopsy surveillance with the intent to detect neoplasia at an early and curable stage. This survey program is burdensome for patients and costly for national health systems. Hence, additional diagnostic strategies, less invasive and with a reduced economic impact, are needed to identify initial alterations suggestive of neoplastic progression.

It is thought that BE-derived EAC arises from the accumulation of genomic alterations that principally

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AT A GLANCE COMMENTARY

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Background

Barrett's esophagus (BE) patients diagnosed with a dysplasia undergo endoscopic biopsies 3 or 4 times a year. This is stressful for patients and of economic impact for health systems. Hence, additional diagnostic strategies, less invasive and with reduced costs, are needed. In this study, longitudinally collected samples of plasma circulating free DNA (cfDNA) from 2 BE patients who progressed to esophageal adenocarcinoma, were analyzed for the presence of genetic alterations suggestive of neoplastic transformation.

Translational Significance

We found that: (i) in early BE carcinogenesis, consistent molecular alterations are detectable in paired cfDNA and formalin fixed paraffin embedded–derived DNA; (ii) cfDNA might represent, better than a single biopsy, the complex heterogeneous architecture of BE and esophageal adenocarcinoma. Thus, liquid biopsy might add clinically important information in the BE scenery, to both the diagnostic assessment of early neoplastic lesions and the post-therapy monitoring.

involve tumor suppressor genes that, in turn, induce genomic instability.[1] Among the involved tumor suppressor genes, several studies underscored the importance of *TP53* alterations, that is, mutations and loss of heterozygosity (LOH), as primary event in the neoplastic progression.[2-6]

BE genetic instability is also the consequence of chronic inflammation associated to gastro-esophageal reflux, obesity, and smoking which are important risk factors for BE development. Sustained inflammation, besides increasing the risk of genomic instability, has been listed among the conditions that promote the release of circulating cell free DNA (cfDNA) in the blood.[7]

In this study, we used the cfDNA, also referred as liquid biopsy, to investigate the presence of neoplastic markers in the plasma of BE patients. Liquid biopsy is, indeed, an emerging approach to detect and follow cancer progression,[8-10] and molecular alterations such as LOH have been found in both the cfDNA and the tumor tissue specimens in different cancer settings.[11,12]

Based on previously reported recurrent alterations in BE and EAC studies,[3,6,13-15] we interrogated longitudinally collected plasma cfDNAs of 2 BE patients that progressed to EAC, for the presence of LOH in chr. 3p14.2, 13q13.2, 17p13.1 and 17p13.2, and mutations in *TP53* gene; the same analyses were also carried out in time-matched biopsy specimens.

MATERIALS AND METHODS

Longitudinal blood samples from 2 BE patients entered in an endoscopic surveillance program, and included in the North-Eastern Italian Registry of BE[16] were collected; time-matched formalin fixed paraffin embedded (FFPE) specimens were also included in the analyses. The study, that was carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki), had the approval of the Ethics Committee of the Veneto Institute of Oncology, Italy (IRB00009272 CE-IOV); written informed consent was obtained via an Institutional consent form.

The QIAamp Circulating Nucleic Acid and the QIAamp DNA Mini kits (Qiagen, Milan, Italy) were used to isolate cfDNA from the plasma and DNA from FFPE specimens, respectively. Genomic DNA was isolated from the patient peripheral blood leukocytes. DNA quantity and quality were determined using the NanoDrop 1000 spectrophotometer (Agilent Technology, Santa Clara, Calif). Neoplastic cellularity in FFPE samples was assessed by microscopic examination and enriched by manually macro-dissecting 8 consecutive 10-μm thick sections. At least 70% of neoplastic component was considered as adequate for the analysis. Nondysplastic Barrett's samples were not subjected to macro-dissection.

LOH was investigated using 4 microsatellite markers: 2 (D17S578, in chr. 17p13.1 and D17S796, in chr. 17p13.2) that map at 0.7 and 1.3 Mbp from *TP53*, respectively, D3S1234 (chr. 3p14.2) internal to *FHIT*, and D13S267 (chr. 13q13.2) at 1.3 Mb from *BRCA2*; *TP53* sequencing of exons 4, 5, 7, and 8 was also performed. Primer sequences and polymerase chain reaction conditions are available on request. Polymerase chain reactions and sequence products were analyzed by capillary electrophoresis using the 3730xl DNA analyzer (Life Technologies, Monza, Italy). All samples were tested in duplicate to assess data reproducibility. LOH was defined as a reduction in one allele compared with the reference (i.e. genomic DNA) of $\geq$40% for cfDNA, and $\geq$35% for FFPE-derived DNA.

RESULTS

Patient B5. The patient was a 61-year-old Caucasian male, presented at our institution with a diagnosis of high-grade intraepithelial neoplasia (i.e. high-grade dysplasia, [HGD]) raised in a long BE segment. An