The use of gastric washes for the identification of DNA mutations in gastric cancer: Implications for liquid-biopsy and patient follow-up

Melissa Pool Pizzi (AC Camargo Cancer Center, Brazil), Maria Galli de Amorim (AC Camargo Cancer Center, Brazil), Adriane Graicer Pelosof (AC Camargo Cancer Center, Brazil), Helano C Freitas (AC Camargo Cancer Center, Brazil), Frederico O Gleber-Netto (AC Camargo Cancer Center, Brazil), Renan Valieris (AC Camargo Cancer Center, Brazil), Israel Tojal da Silva (AC Camargo Cancer Center, Brazil), Maria Dirlei Begnami (AC Camargo Cancer Center, Brazil), Felipe JF Coimbra (AC Camargo Cancer Center, Brazil), Diana Noronha Nunes (AC Camargo Cancer Center, Brazil), Emmanuel Dias-Neto (AC Camargo Cancer Center, Brazil)

BACKGROUND: Gastric adenocarcinomas (GAs) are associated with high mortality rates, especially when the disease is diagnosed at a late stage. Unfortunately, when the first symptoms appear the patient usually presents a more advanced disease, which leads to worse prognosis. Recent sequencing efforts of gastric tumor samples have characterized the mutational landscape of these lesions. These data not only suggested the existence of mutational profiles that characterize distinct molecular GA-subtypes, but it also provides a list of more common mutations that may impact the diagnosis and the prognosis of the different subtypes. If one has a catalog of tumor-derived mutations, it is plausible to assume that it can be used for the identification of a tumor-specific mutation with diagnostic implications. This is of particular interest in a scenario where endoscopic biopsies are not representative of the full mutational diversity of a tumor. Moreover, gastric biopsies are invasive and expensive procedures that in some situations cannot recover significant amounts of tumor material. In this sense, the detection of free DNA in body fluids holds the promise to be an important tool capable of mirroring the tumor mutational heterogeneity and to represent tumor-specific mutations with diagnostic and follow-up implications. HYPOTHESIS: The detection of tumor-specific mutations by sensitive methods might have diagnostic implications and be useful for disease monitoring during and after treatment, especially for early detection of recurrent disease. METHODS: We performed deep sequencing of all exons of the TP53 gene, the most mutated gene in Gas (≅ 40% to 70%), using the Ion Proton platform. DNA was obtained from 29 GA biopsies and their corresponding 29 gastric washes. All samples were derived from untreated patients diagnosed at the AC Camargo Cancer Center. Single nucleotide variations (SNV) in TP53 gene were evaluated in leukocytes, tumor biopsies and gastric washes and the patterns of alterations between these samples were compared for each patient. Mutations were evaluated using the variantCaller (v5.0.2.1) plugin. RESULTS: We generated a mean of 6,6 million reads per sample, resulting in an average coverage >13,000X. Nine patients (31%) were diagnosed with GAs of the intestinal type and the remaining 20 (69%) presented the diffuse type. TP53 mutations were observed in biopsies of 12 patients (41,4%, being 56% of the intestinal-type and 35% of the diffuse-type), and 75% of these were non-synonymous. For 8 patients (66%) the same mutation found in the primary tumor was also detected in the gastric washes. Detection of tumor-derived mutations in the gastric washes was slightly higher in diffuse-type (71% versus 60%). Interestingly, for another 4 cases, TP53 mutations were detected in gastric washes, but did not appear in the respective tumor biopsies. These results show for the first time the availability of tumor-derived DNA in gastric washes, a finding that highlights the opportunity of liquid biopsies to be performed in this material, with possible implications for diagnosis, treatment monitoring and early detection of tumor recurrence.

Financial support: FAPESP, CAPES and CNPq