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LETTER TO THE EDITOR Open Access

Genomics and epidemiology for gastric adenocarcinomas (GE4GAC): a Brazilian initiative to study gastric cancer

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Abstract

Gastric cancer (GC) is the fifth most common type of cancer worldwide with high incidences in Asia, Central, and South American countries. This patchy distribution means that GC studies are neglected by large research centers from developed countries. The need for further understanding of this complex disease, including the local importance of epidemiological factors and the rich ancestral admixture found in Brazil, stimulated the implementation of the GE4GAC project. GE4GAC aims to embrace epidemiological, clinical, molecular and microbiological data from Brazilian controls and patients with malignant and pre-malignant gastric disease. In this letter, we summarize the main goals of the project, including subject and sample accrual and current findings.

Keywords: Gastric cancer, Epidemiology, Microbiome, Whole exome sequencing, Genomic ancestry, Liquid biopsy, Hereditary gastric cancer, Database

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The global distribution of gastric cancer (GC) is heterogeneous, a consequence of worldwide socio-economic disparities as well as of the human-diversity in terms of ancestry and cultural habits, including diet. This heterogeneity is not limited to global incidence but also embraces treatment response, clinical and pathological presentation, and the mutational landscape. This leads to frail translation of the still limited findings from US/Europe/Asia to other countries, including highly admixed and least-studied populations. In an attempt to reduce the GC knowledge-gap, the GE4GAC project, supported by FAPESP (14/26897–0), was implemented in 2016 at the A.C.Camargo Cancer Center (ACCC) in São Paulo, Brazil. Collaborations are underway with the National Cancer Institute (NIH, USA); Instituto Nacional de Enfermedades Neoplásicas (Peru) and both the VOGAS (www.vogas.eu) and the StoP (www.stop-project.org) consortia. Here, we present our cohort/sample accrual (May 2019), results and aims.

Clinical aspects

At the ACCCC ~ 130 GC-patients are diagnosed/year. Upfront surgery is performed in ~ 15% of early-disease cases. Patients with cT3/T4 tumors and N1,2,3 (~ 50%) usually receive perioperative chemotherapy and surgery (~ 70 gastrectomies/year), after staging laparoscopy. Most cases are discussed in weekly multidisciplinary tumor boards.

Complete pathologic-response is seen in ~ 12% of patients. Median follow-up so far was 41 months, and the median survival since neoadjuvant chemotherapy was 73 months. Intention-to-treat analysis showed a 5-year overall survival of 65%. Clinical research includes the identification of surgical prognostic factors [1, 2] and the factors that impact outcome [3–5]. Patients are also invited to join the GE4GAC to investigate different aspects, such as the factors that contribute to GC development in Brazil, genes relevant for familial GC, predictors of neoadjuvant chemotherapy response and molecular variants that may indicate prognosis. Controls from the endoscopy-sector and the cancer-prevention campaign also participate (no-cancer) [6].

Epidemiology

We investigate associations between GC and demographic characteristics, dietary habits, and lifestyle primarily in subjects living in three geographic areas of Brazil: i) São Pãulo-SP (Southeast), ii) Belém-PA (North) and iii) Fortaleza-CE (Northeast). A total of 1121 subjects (Feb-2016 to May-2019), aged 35–74 years were interviewed, including GC-cases ($N = 412$; C16/ICD-O3) and two control groups including subjects recruited during the cancer-prevention campaigns at ACCCC ($N = 153$), or from the Endoscopy Department (no-cancer; $N = 556$).

Basic and translational GC research

Collected samples ($N = 2856$) include whole-blood, gastric-fluids, biopsies, saliva, and leukocytes, derived from > 400 cases+controls. These are used to investigate:

i) Gastric Microbiota: We investigate possible links between microbiota and GC, including tumor subtypes/location and neoadjuvant treatment/response. 16SrRNA sequencing, chosen due to elevated human:eubacteria ratios found in our samples, has been performed for ~ 460 samples, indicating ~ 50 bacteria present in biopsies and 2X-more for gastric-fluids. Reduced microbiota richness/diversity in cases was observed compared to controls. Shotgun-metagenomics and metatranscriptomics will be performed for selected samples, together with bacterial cultivation, to determine dead vs. alive bacteria. Virome and fungal populations will also be evaluated. qPCR EBV-analysis ($N = 400$) showed 12.7% EBV+ in GC-cases and 2.9% in controls. We recently showed a possible new mechanism of EBV-carcinogenesis where EBV-infection triggers APOBEC3 over-expression and an APOBEC-mutation signature [7].
ii) Whole exome sequencing (WES) and RNA-Seq: These will give comprehensive views of genome-wide alterations pertinent to our population and clinical interests, including immune infiltrates. We aim to have WES for ~ 400 patients and RNA-Seq mainly to support treatment-response studies.
iii) Genomic ancestry: Using a set of ~ 130,000 Ancestry Informative Markers [8] we found that the average cohort participant has a predominance of Mediterranean (50%), Northern-European (13.5%) and Southwest-Asia (12.5%) ancestries, followed by Sub-Saharan African (4.3%) and Native-American (3.8%) (unpublished). Genomic ancestry will be determined for all subjects and correlated against biological and demographic aspects, such as microbiota, diet, mutation profiles and treatment outcome.
iv) Liquid-biopsies: We evaluated the presence of cell-free tumor-derived mutations in gastric washes (GW) collected during endoscopy, comparing mutation levels with biopsies and plasma. Deep sequencing showed enhanced detection when plasma and GW are evaluated together. GW has been shown to carry mutations not found in biopsies, suggesting tumor heterogeneity/cancerization-field while providing a tool for monitoring treatment-response and disease-recurrence [9].

Other liquid-biopsies approaches investigated are Circulating Tumor Cells (CTCs) and Extracellular Vesicles

(EVs). EVs-studies have been initiated in-vitro, as a means to study chemotherapy-resistance. Hundreds of patient-derived samples have been collected for further studies, including EV-RNA-Seq [10–12] and proteomics [13]. The prognostic-value of CTCs in GC was studied in 88 samples, collected from 55 GC-patients (before/after neoadjuvant-chemotherapy) [14]. A clinical trial of trastuzumab given to metastatic GC patients with HER2-negative tumor tissue but HER2 FISH-positive CTCs will start soon.

v) Hereditary GC (HGC): Germline pathogenic variants were found in *CDH1, MUTYH, TP53,* and *ATM* in 21% of HGC-suspect patients. Novel GC-predisposing genes are under study by WES.

RedCap: a structured database

Data from all enrolled subjects are securely kept in RedCap, a web-based application that currently holds the records from 401 controls and 440 GC-cases, including 1632 types of metadata, such as lifestyle ($N = 845$), diet ($N = 629$) and clinicopathological ($N = 41$) variables. The collection of massive amounts of data in a single unified database is a key aspect of GE4GAC, a project that is open for worldwide collaborations.

Abbreviations

ACCCC: A.C.Camargo Cancer Center; CTCs: Circulating Tumor Cells; EBV: Epstein-Barr virus; EVs: Extracellular Vesicles; FAPESP: Fundação de Amparo à Pesquisa do Estado de São Paulo; GC: Gastric Cancer; GE4GAC: Genomics and Epidemiology for Gastric Adenocarcinomas; GW: Gastric washes; ICD-O3: International Classification of Disease for Oncology; OTU: Operational Taxonomic Unit; WES: Whole Exome Sequencing

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Authors' contributions

Manuscript writing and GE4GAC coordination: ED-N. Epidemiology: MPC, SVP, VFC, RB, CRN, GPBB, ACCP, CMG, MAF, MSA, TVM, MSB, DMMC, LCC, AMB, AFPLR, LLLS, JCP, TT, PEA, VS, MC, ROS, HBA, AKMA, SVS, DTS, LOM; Bioinformatics: ITS, AMT, BSM, RV, AD, RB, RDCD, IB, MNPS, JCS; EV-studies: VRM, DRC; Familial/early-onset disease: DMC, KMS, GTT; CTC-studies: LTDC, EAA, ABC, BCTF; Immunological studies: KJG, TM; Clinical/Pathological studies: FJC, WLCJr, HCF, LGVC, SD, CALM, VHFJ, RR, LCLC; Endoscopy and sample collection: AGP, CZS; Genomics:TFB, MGA, JMS, GEA, GPB, HCF, LFA, MSS, IKTMT, EE, DNN, ED-N; Microbiome: TFB, MSS, GEA, AMT, DTS, LOM, BSM, AK, SD, PPA, IKTMT, ITS, DNN. Research nurse: LLSA; Data manager: FIAV. All authors have read and expressed their consent to publish this letter.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

The study presented here was approved by the institutional review board (Comitê de Ética em Pesquisa - CEP) of the A.C.Camargo Cancer Center (protocol 2134/15), and all participants signed an approved informed consent form.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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