Brazilian intestinal gastric cancer displays a common molecular signature worldwide

Santos E.C[1]; Binato R[1]; Boroni M[2]; Demachki S[3]; Assumpção P. P[3]; Abdelhay E.[1]

[1]Laboratório de Células Tronco, Centro de Transplante de Medula Óssea - CEMO,INCA; [2]Laboratório de Bioinformática e Biologia Computacional, Instituto Nacional de Câncer (INCA), Rio de Janeiro, RJ, Brazil; [3]Núcleo de Pesquisa em Oncologia, Hospital João de Barros Barreto, Universidade Federal do Pará.

INTRODUCTION

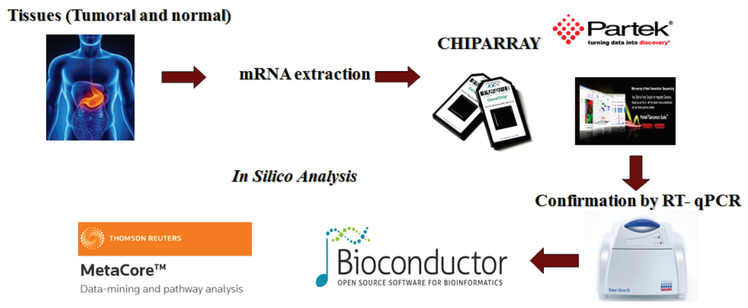
Gastric cancer (GC) is the fourth most common cancer in the world. In Brazil it is the fourth most common in males and the sixth in females, and in 2017, 13,000 new cases for men and 7,600 for women were expected. GC is a multifactorial disease comprehending lifestyle, aging, genetic, socioeconomic factors, and also infection by *Helicobacter pylori*, which has been attributed in 80% of the cases and Epstein-Barr virus (present in 6-10% of cases). One of the great problems of GC is the late disease detection caused by nonspecific symptomatology in early stages, which is associated with poor prognosis. According to Lauren classification, the adenocarcinoma presents two types: intestinal (well differentiated with cohesive neoplastic cells) and diffuse (poorly differentiated with infiltration and thickening of the stomach wall). Innovative technologies have been used in the last years to identify alterations in gastric cancer cell biology. Several genetic abnormalities, such as aberrant genes copy number variation, microRNAs, and long noncoding RNAs were identified as possible biomarkers in these studies. However, the molecular mechanisms leading to gastric cancer and those responsible for its progression are not clearly understood. Moreover, almost all studies data were described from Asia or Central America populations. Thus, much of the information in the literature cannot be considered general for all populations.

OBJECTIVE

In order to better understand the disease, the aim of this work was to analyze by microarrays the gene expression profile of brazilian intestinal gastric cancer patients and compare to other populations worldwide and also try to identify genes that could predict overall survival.

METHODOLOGY

We performed chip arrays to compare the gene expression profiles of tumor samples from Brazilian patients with intestinal gastric cancer with those of non-tumor tissue from the same patients (control). RNA samples were obtained using *RNeasy Mini kit (Qiagen, CA, USA)*. RNA were prepared and hybridized to GeneChip Human Gene 1.0 ST Arrays (Affymetrix, CA, USA). The data were analyzed using Partek® and differentially expressed genes with a 5-fold change were used as criteria to define overexpression or downregulation. To confirm the results, we performed a quantitative PCR (RT-qPCR) of some overexpressed and downregulated genes. We also performed an unsupervised analysis with microarrays from different studies worldwide using this Brazilian molecular signature. *In silico* analysis using the MetaCore™ software (GeneGO Inc., Encinitas, CA) was carried out to access the processes and pathways associated with these data. Moreover, we conducted a survival analysis based on the 38 genes expression from our molecular signature to verify if some genes could influence overall survival.



RESULTS

Gene expression profile

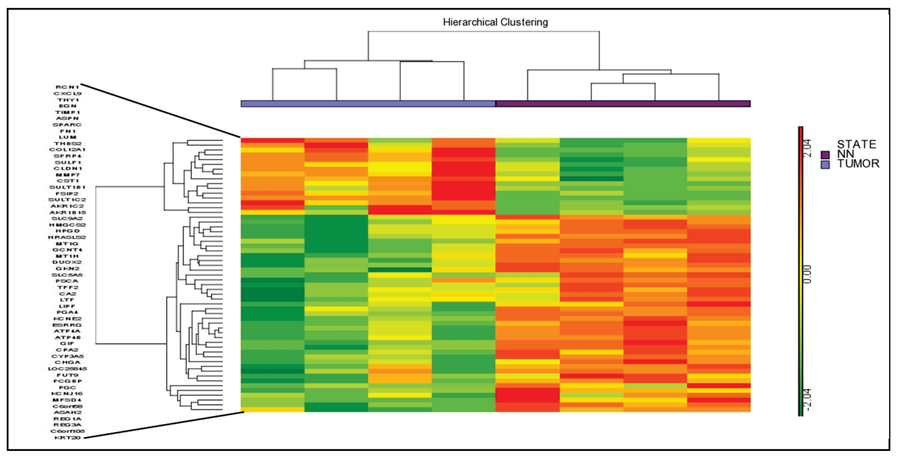


Figure 1. Hierarchical clustering of the 57 differentially expressed genes identified by chip array assay. The results showed a common molecular signature for tumor tissues from intestinal gastric cancer compared to non-tumor tissue counterpart.

Unsupervised analysis

Table1: Microarray data from other studies used in unsupervised analysis.

Study - GEO acession	Microarray-platform	Nationality	Histologycal type
GSE15456	Affymetrix Human Genome U133A Array	United Kingdom	Intestinal
GSE15459	Affymetrix Human Genome U133 Plus 2.0 Array	Singapore	Intestinal
GSE19826	Affymetrix Human Genome U133 Plus 2.0 Array	China	Non Tumor
GSE22377	Affymetrix Human Genome U133 Plus 2.0 Array	Germany	Intestinal
GSE29272	Affymetrix Human Genome U133A Array	China	Intestinal Non Tumor
GSE37023	Affymetrix Human Genome U133A Array	Several Cohorts	Non Tumor
GSE38749	Affymetrix Human Genome U133 Plus 2.0 Array	Brazil	Intestinal
GSE47007	Affymetrix Human Genome U95 Version 2 Array	Japan	Intestinal
GSE57308	Affymetrix Human Genome U133 Plus 2.0 Array	China	Intestinal
GSE62254	Affymetrix Human Genome U133 Plus 2.0	Asian Cancer Research Group cohort	Intestinal

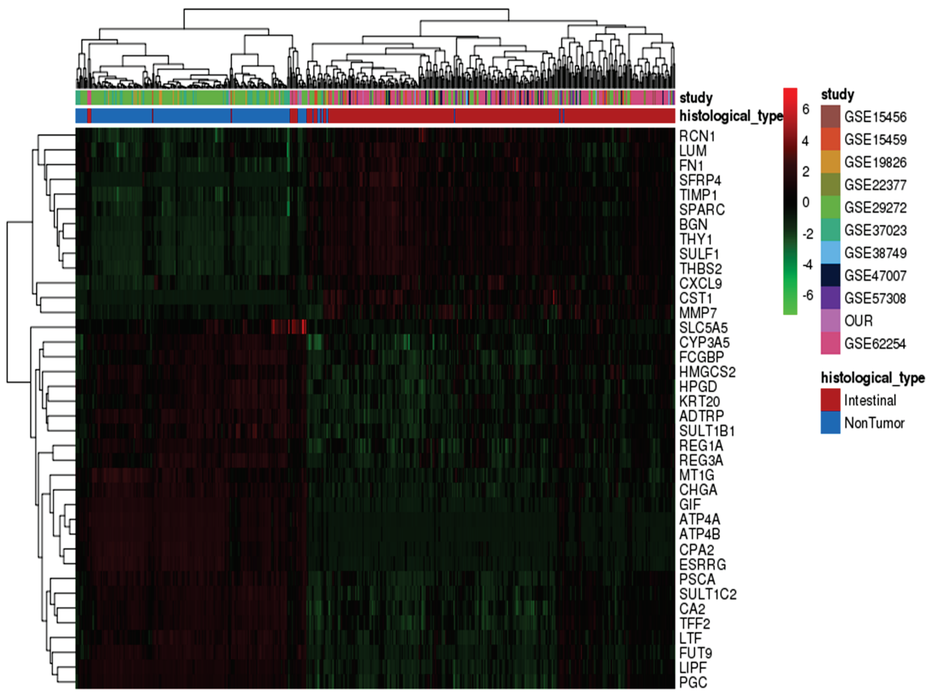


Figure 3. Unsupervised analysis of the differentially expressed genes found in brazilian intestinal gastric cancer in different populations samples. Hierarchical clustering of samples using 38 genes differentially expressed between non-tumor and tumor samples from different studies. Each row represents a gene, and each column represents a sample. The expression level of each gene in a single sample is relative to its median abundance across all samples and is depicted according to a colour scale shown at the right. Red and green indicate expression levels above and below the median, respectively. The magnitude of deviation from the median is represented by the colour saturation. Dendrograms of samples (above matrix) and genes (to the left of matrix) represent overall similarities in gene expression profiles. For samples, blue boxes represent non-tumor condition (n=190), and red boxes represent cancer condition (*n*=312). Colored boxes represent datasets from different studies showed in table 1.

Microarray data validation

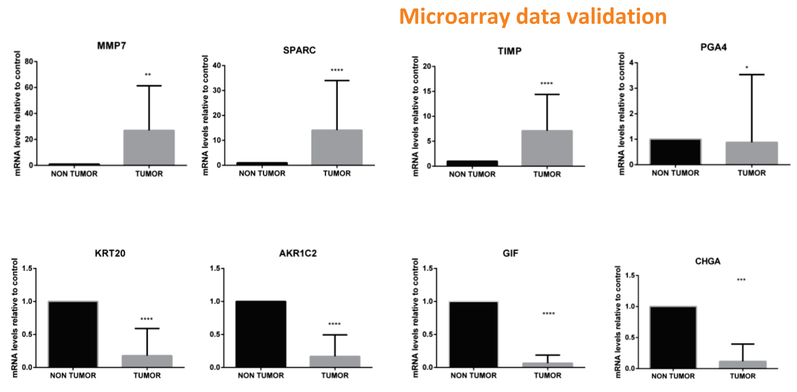


Figure 2. RT-qPCR to validate the chip array assay results. To confirm the obtained chip array results, RT-qPCR was used to analyze some differentially expressed genes using a larger number of Bazilian patient samples to determine changes in mRNA expression levels after normalization to Actin and *GAPDH*. RT-qPCR analyses of *MMP7*, *SPARC* and *TIMP1* (overexpressed in patients with intestinal gastric cancer) and *PGA4*, *KRT20* , *AKR1C2*, *GIF* and *CHGA* (downregulated in patients with intestinal gastric cancer) confirmed the chip array assay results and the common molecular signature that was able to discriminate all tumor tissues from all intestinal-type gastric carcinoma patients from non-tumor control tissue. *p<0.05; **p<0.01.

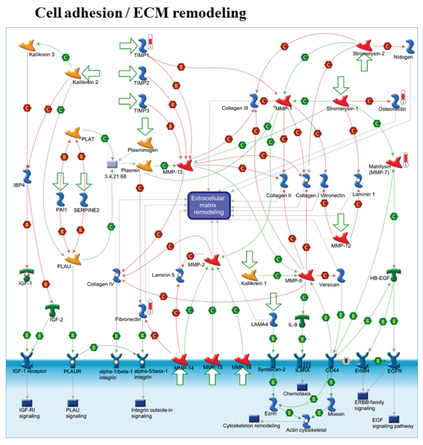
In silico analysis

Table2: Processes related to the 38 common genes differentially expressed in intestinal gastric cancer.

Functional Enrichment Analysis [a]	Gene [b]	
	UP	DOWN
Extracellular Matrix Remodeling	TIMP1, MMP-7, FN1, SPARC, LUM	
Gastrin in differentiation of the gastric mucosa		REG1A, CHGA, TFF2
Stimulation of gastric acid secretion		ATP4A, ATP4B, CHGA
Cell adhesion_Cell-matrix interactions	LUM, TIMP1, MMP-7, FN1, BGN	REG3A
Inflamation	TIMP1, CXCL9, FN1	REG3A

a. Enrichment analysis was performed using Metacore™
b. Gene symbols from 38 genes found in our unsupervised analysis which were identified to be significantly up- or down-regulated.

Cell adhesion / ECM remodeling



Development Gastrin in differentiation of the gastric mucosa

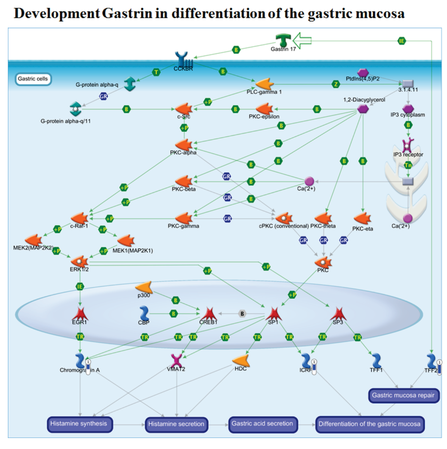


Figure 4: *In silico* analysis using MetaCore™ *software* (GeneGO Inc., Encinitas, CA) to verify the processes and pathways associated with the differentially expressed genes. These genes are primarily involved in processes related to Extracellular Matrix Remodeling and Differentiation of Gastric Mucosa.

Overall survival analysis

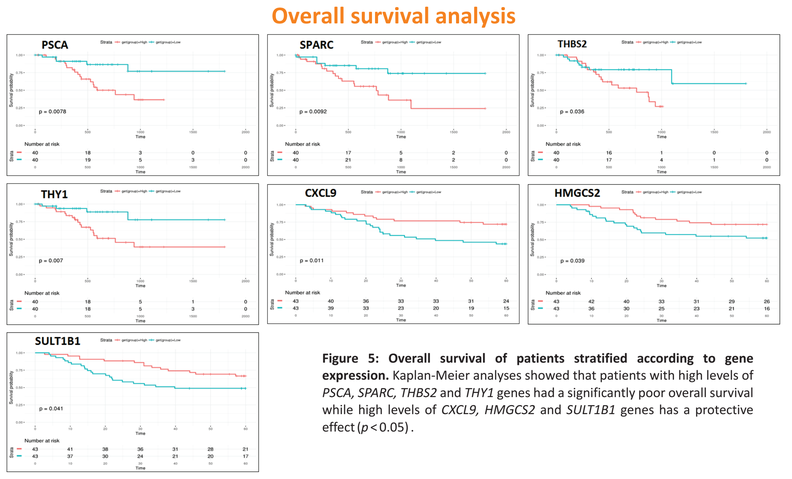


Figure 5: Overall survival of patients stratified according to gene expression. Kaplan-Meier analyses showed that patients with high levels of *PSCA, SPARC, THBS2* and *THY1* genes had a significantly poor overall survival while high levels of *CXCL9, HMGCS2* and *SULT1B1* genes has a protective effect ($p < 0.05$) .

CONCLUDING REMARKS

Altogether the results showed a molecular signature for all intestinal gastric cancer compared to nontumor counterpart regardless of the population, and this molecular signature could be related to important processes in cell homeostasis. Moreover some of these genes could predict overall survival in patients with intestinal gastric cancer.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA