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REVIEW ARTICLE

Gastric cancer and the omics

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Abstract

Gastric cancer is a fatal process whose risk factors include infection by Helicobacter pylori, pernicious anemia, nitroso compounds, alcohol abuse, cigarette smoking, and male gender. Endoscopic surveillance has defined the histological progression of premalignant to malignant lesions. Nevertheless, gastric tumors exhibit distinct histologic variations, clinical behaviors, and treatment responses. This "intra and interpatient heterogeneity" has obliged us to search for key principles governing gastric cancer evolution. Advanced bioinformatics programs, DNA microarray technology, and functional genomics have helped to integrate the structure, function, and dynamics of biological molecules expressed or secreted by cancer epithelial cells that have modified the classification of gastric cancer.

Keywords: *Gastric cancer. Omics classification. GC diversity. Bioinformatics.*

Cáncer gástrico y las ómicas

Resumen

El cáncer gástrico es un proceso fatal cuyos factores de riesgo incluyen la infección por Helicobacter pylori, la anemia perniciosa, la ingesta de compuestos nitrosos, el abuso de alcohol, el tabaquismo y el sexo masculino. El seguimiento endoscópico ha definido la progresión histológica de lesiones premalignas a lesiones malignas. Sin embargo, los tumores gástricos exhiben diferentes y muy variadas variaciones histológicas, comportamientos clínicos y respuestas a tratamiento. Esta gran heterogeneidad intrapaciente e interpaciente ha obligado a la búsqueda de principios reguladores que gobiernen la evolución del cáncer gástrico. Los programas avanzados de bioinformática, la tecnología de microarreglos de ADN y la genómica funcional han ayudado a integrar la estructura, función y dinámica de moléculas biológicas expresadas o secretadas por las células epiteliales cancerosas que han modificado la clasificación del cáncer gástrico.

Palabras clave: *Cáncer gástrico. Clasificación ómicas. Diversidad del cáncer gástrico. Bioinformática.*

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Introduction

Gastric cancer is a leading cause of global cancer morbidity and mortality[1]. It exhibits high levels of histologic, transcriptomic, and epigenomic variation with distinct clinical behaviors and treatment responses[2] but the understanding of gastric tumor biology and the key principles governing gastric cancer evolution are far from being grasped. The Correa Pathway of gastric carcinogenesis describes the histological progression of normal gastric epithelia toward gastric cancer[3] but the emergence of DNA microarray technology and the establishment of functional genomics have modified the classification of gastric cancer. The characterization of this new way to study and integrate structure, function and dynamics of biological molecules has been incorporated in the omics concept. The goal of the omics concept is to screen cell changes involved in cancer hallmarks using techniques such as next-generation sequencing and mass spectrometry techniques. The initial goal is to identify disturbances of genes associated with cell growth control such as oncogenes, tumor suppressor and carekeeper genes, to determine (a) genomic instability whether there are chromosomal balanced structural changes, nonreciprocal structural changes, or altered genetic code of certain genes, or (b) epigenomic instability secondary to DNA methylation-associated to transcriptional silencing of genes and/or activation of oncogenes-, histone modification - that determines gene activation or repression-, or the effect of non-coding RNA whose role depends on their interaction with RNA, or DNA, or protein. Several of these changes to the genetic material can co-occur. Nevertheless, gene regulation in cancer is a hot topic. The branches of research that the concept omics refers to include various biology disciplines that overall try to identify, characterize, and quantify biological molecules that are involved in the structure, function, and dynamics of a cell or a tissue. The objects of study encompass: (1) genomics, the integration of all the disciplines related to the study and application of genomes, (2) epigenomics, the study of the reversible modifications on the genome that affect gene expression without altering the DNA sequence, (3) transcriptomics, the analysis of messengers and non-coding RNA transcripts produced by the genotype at a given time, (4) proteomics, the large-scale study of protein structure and function, (5) metabolomics, the study of the chemical processes involving metabolites, small molecule substrates and intermediates, (6) secretomics, the analysis of all the secreted proteins of a cell, it is considered as a type of proteomics, (7) interactomics, the study of the whole set of molecular interactions in a biological system, (8) metagenomics, the study of the structure and function of entire nucleotide sequences recovered from organisms, (9) lipidomics, the study of the structure, function and interaction of lipid molecules with other lipids and proteins, (10) glycomics, the study of the biological functions of any carbohydrate structure produced by a cell or tissue under specified conditions, and (11) immunomics, the study of the detailed map of immune reactions of a host interacting with a foreign antigen.

Intricacy of histological classifications

As early as 1965 Lauren proposed a histological classification that divides gastric cancer into intestinal type (mainly associated with *H. pylori*), diffuse type, and undetermined type[4]. The incidence of diffuse-type gastric cancer has increased. Nevertheless, gastric cancer is a heterogeneous disease that must be stratified not only by histopathological differences. In 2010, the World Health Organization introduced a classification that recognizes four major histological patterns: tubular, papillary, mucinous, and poorly cohesive, but GC histological classification has followed a tortuous road because anatomical location, tumor size, growth, protrusion, presence of polypoids, and mucosal invasion have been considered in many different classifications[5]. The successful use of cancer genomics programs and transcriptomic analysis has allowed for new gastric cancer classifications and categories beyond their histological definition.

Influence of omics in the classification of gastric cancer

The *Cancer Genome Atlas* considers four molecular subtypes, (1) the Epstein-Barr virus positive (EBV^+), (2) the microsatellite unstable (MI), (3) the genomically stable (GS), and (4) the chromosomal unstable (CIN). The genetic alterations that define the EBV^+ subtype comprise PI3KCA mutation, DNA methylation, PD-L1/2 overexpression and activated immune systems; the hallmarks of the MI subtype are high mutation burden, ARID1A mutation, DNA hypermethylation and activated mitosis, whereas those of the GS subtype are CDH1 and RHOA mutation, Cldn18-ARHGAP fusion, inactivated cell adhesion and histological diffuse type. Finally, the hallmarks for the CIN subtype, which is the most frequent subtype, are TP53 mutation and RTK-RAS amplification. Interestingly most of the poor prognoses diffuse type gastric cancer corresponds to the GS subtype.

The Asian Cancer Research Group (ACRG) has identified four subtypes of gastric cancer linked to distinct patterns of molecular alterations and associated with distinct clinical outcomes: microsatellite-unstable tumors, microsatellite-stable tumors with epithelial-to-mesenchymal transition, microsatellite-stable TP53 positive tumors, and microsatellite stable TP53 negative tumors[6]; P53 activation is based on the detection of the negative regulator CDKN1A and MDM2.

The *Centre for Computational Biology of Duke-National University of Singapore*[7] evaluated the gene expression patterns of gastric adenocarcinomas as they show great heterogeneity expression patterns. The group search for a robust classification of gastric cancer identified three different molecular subtypes with distinctive genomic and epigenomic properties: (1) the proliferative subtype that has high levels of genomic instability, high-level TP53 mutations, and DNA hypomethylation, (2) the metabolic subtype that showed high activity of a pathway associated to the spasmolytic-polypeptide- expressing metaplasia considered as an intermediate in the development of gastric adenocarcinoma and is more sensitive to 5-fluorouracil, and (3) the mesenchymal subtype that contain features of cancer stem cells such as high mRNA levels of N-cadherin, low levels of E-cadherin, activation of transforming growth factor (TGFβ) or vascular endothelial growth factor pathways and are sensitive to PI3K-AKT-mTOR pathway inhibitors. The differences between subtypes were not associated with significant survival variances but the classification might be used to select specific treatments. Interestingly, the comparison of the ACRG and TCGA classifications points to similarities between the different subtypes: MSI with EBV^{+}, MSS/EMT with GS, MSS/$TP53^{-}$ with MSI, and MSS/$TP53^{+}$ with CIN. The analysis of genomic and proteomic data as well as signaling pathways[8] identified two distinct gastric cancer molecular subtypes, (1) the mesenchymal phenotype that shows high genomic integrity, low mutation rates, microsatellite stability and highly activated EMT transition, sensitive to inhibition of IGF1/IGF1R and TGFβ signaling pathways but with markedly poor survival and resistance to standard chemotherapy; interestingly, the diffuse histological phenotype was more common among the mesenchymal phenotype tumors, and (2) the epithelial phenotype that display low genomic integrity but is associated with better survival rates and sensitivity to chemotherapy.

It is worth noticing that in these different gastric cancer subtype classifications there is a great variety of genomic landscapes that derive from the differences in epigenetic mechanisms - DNA methylation, histone modification, noncoding RNAs-or driver gene, such as AR, MYC, or PPARA mutations derived from exposure to pharmaceutical or toxic chemicals, diet, stress, exercise, microbiome, disease exposure, and drug abuse of the different population samples that were analyzed. But it seems that the TGFβ signaling pathway and most importantly, the epithelial-to-mesenchymal process are predominantly affected in all the classifications. Translating proteomic subtyping into clinically proficient early detection markers and/or treatments is an imperative and critical research direction. Table 1 shows some of the main associated genes according to classification.

Genomics and epigenomics

An epigenomic histone modification profile of gastric cancer samples revealed that cancer-relevant gene expression is influenced by enhancer[9] differences in genomic copy number and that HNF4a, a nuclear transcription factor that controls the expression of several genes is a master trans-acting factor associated with cancer heterogeneity[10].

A recent analysis of transcriptomic profiles of primary gastric cancers derived into a consensus mesenchymal-subtype gastric cancer (Mes-GC) classifier[11] where TEAD1 (a transcriptional enhancer factor) is a master regulator of Mes-GC enhancers, especially NUAK1 kinase (a serine/threonine-protein kinase involved in cell proliferation). The results determined that TEAD1 inhibition and combinatorial NUAK1 inhibition/cisplatin represent a therapeutic target.

Proteomics-based classification

Proteomics has been successfully used to classify the diffuse type as the most severe histological type of gastric cancer that has poor clinical outcomes. The gene ontology analysis of gastric cancer tumor proteomics indicated that the altered genes were significantly enriched in EMT, cell cycle, DNA replication, p53 signaling, and inflammatory response pathways whereas normal nearby tissue was enriched with fatty acid metabolism, oxidative phosphorylation, and amino acid metabolism pathways. The use of consensus clustering methodology helped in the identification of three different subtypes based on differentially expressed proteins and distinctive pathway enrichment and clinical outcome: (1) the PX1 cluster that exhibits dysregulation in the cell cycle, (2) the PX2 cluster that

Table 1. Genes associated with histologically/molecular classified subtypes

Classification	Subtype	Major associated genes
Lauren	Intestinal	FUT, LGALS4, CDH17
Lauren	Diffuse	AURKB, ELOVL5
Lei	Proliferative	Decreased TP53 mutations
Lei	Metabolic	Increased TP53 mutations
Lei	Mesenchymal	Increased TP53 mutations
*CGA	EBV- positive	PIK3CA, JAK2, PDL1/2, BCOR
*CGA	Microsatellite instable	PIK3CA, ERBB2/3, EGFR, PDL1, MLH1, TP53
*CGA	Genomic stable	CDH1, RHOA
*CGA	Chromosomal unstable	SMAD4, APC, TP53, RTK-RAS
**ACRG	Microsatellite unstable high	ARID1A, MTOR, KRAS, PIK3CA, ALK, PTEN
**ACRG	Microsatellite stable/Epithelial-mesenchymal transition	CDH1
**ACRG	Microsatellite stable/TP53+	APC, ARID1A, KRAS, PIK3CA, SMAD4
**ACRG	Microsatellite stable/TP53-	ERBB2, EGFR, CCNEI, CCND1, MDM2, ROBO2, GATA6, MYC

*CGA: cancer genome atlas; **ACRG: Asian cancer research group

on top of the dysregulation of the cell cycle features an additional epithelial-to-mesenchymal process, and (3) the PX3 cluster that is enriched in immune response proteins, has the worst survival and is insensitive to chemotherapy.

The intent to reproduce molecular classifications using immunohistochemical analysis and in situ hybridization has proved to be useful as the results have corroborated the effectiveness of this approach. The results show that the evaluation by both methods of EGFR, HER2, TP53, EBV, E-cadherin, MLH1, and microsatellite instability help to classify biological and clinically different gastric cancer subgroups. The relevance of using immunohistochemistry to complement omics analysis has been established. Using a similar approach subset within the defined molecular subtypes has been identified. Like claudin-6, a protein not expressed in normal gastric epithelia and strongly associated with greater gastric cancer invasiveness and metastatic capacity[12] which has been proposed as a subcategory of the CIN molecular subtype of gastric cancer. CIN gastric cancer overexpressing claudin-6 show higher mutations in TP53, MIEN1, STARD3, PGAP3, CCNE1, MAGEA9b, and APOA2 genes[13].

The great genomic diversity found that in gastric cancer bioinformatics analysis is further exemplified by particular analysis of signaling and metabolic pathways made considering a single protein for example claudin 6 high expression where the main major disrupted pathways are complement and coagulation cascades (3.3-23), cholesterol metabolism (1.9-12), fat digestion and absorption (1.8-7), and many others[13].

Secretomics-based classification

The latest comprehensive multi-omic analysis of gastric cancer malignant ascitic fluid samples classification stratified ascites-disseminated gastric cancer metastases into two distinct molecular subtypes: one displaying active super-enhancers at the ELF3, KLF5, and EHF loci, and a second where the transcriptional enhancer factor TEF-1 is highly expressed and TGFβ pathway is activated through SMAD3 (Table 1)[14].

Despite differences in histology and outcomes between the intestinal and the diffuse subtypes proteomic and phosphoproteomics analysis allowed the identification of specific signatures between both subtypes[15]. A total of 4,846 proteins were identified in the diffuse type, interestingly 255 were overexpressed and 372 were underexpressed, whereas in the intestinal subtype, a total of 7,448 proteins were identified but only 15 were overexpressed and 56 were underexpressed; a further pathway analysis showed that a canonical pathway that favors the accumulation of acetaldehyde,

associated to gastric cancer development due to its effect on the DNA, was the most involved in the intestinal subtype. *H. pylori* infection is mainly associated with the intestinal subtype because of its enhanced production of acetaldehyde.

Phosphoproteomics

The analysis of phosphorylated amino acid residues in proteins from biological species has evolved as kinase inhibition is now involved in cancer therapy. Gastric cancer phosphorylation landscapes have elucidated signaling pathways associated with somatic mutations based on mutation-phosphorylation correlations[16]. The phosphorylation landscape of 28,000 diffuse gastric cancer phosphorylation sites identified 445 upregulated phosphorylated sites associated with cell-cycle pathways cell-cell adhesion, DNA repair and mRNA splicing pathways but consensus clustering identified three clusters, Ph1, Ph2, or Ph3, associated with distinct clinical outcomes[17]. The Ph1 subtype showed upregulated rRNA processing and RNA polymerase II promoter activity, the Ph2 subtype upregulated DNA metabolism and DNA repair but lost gastric acid secretion, the Ph3 subtype upregulated chromosome segregation and lost cell-cell interaction and communications.

Glycomics

Analyze the structure and function of glycans in biological systems. O-glycan alterations in gastric cancer tissue are the result of glycosyltransferases accessibility and/or availability of sugar-nucleotide precursors amongst many others. Altered glycosylation is a hallmark of malignant transformations that contributes to disease outcomes[18]. Aberrant glycosylation including an increase in overall sialylation of sialyl Lewis x and sialyl Lewis a antigen as well as an increase in terminal a2,6-sialylated structures in truncated O-linked and N-linked glycans has been reported in many different cancers. The same modifications are reported in gastric cancer although the differences can not specifically differentiate between subtypes[19] some reports indicate that mucin-associated sialylated antigens Sialyl Lewis^{a} $^{and\ x}$, as well as Sialyl Tn expression in the diffuse subtype, have a worse prognosis[20].

Secretomic and immunomics analysis has helped untangled the complexities of tumor microenvironments. It has long been recognized that cancer and stromal cells, mainly fibroblasts, interact in the cancer microenvironment by secreted proteins (TGFβ, PDGF, FGF-2) through autocrine and paracrine pathways. The analysis of the functional secreted molecules involved in gastric cancer showed that the secretion of growth and differentiation factor 15 (GDF15), a molecule only expressed in fetal tissue and placenta, is significantly higher in the diffuse subtype gastric cancer[21,22]. GDF15 has an immunoregulatory function and it is now considered as an immune checkpoint thus becoming a target for cancer immunotherapy. The FGFR1 and 2 proteins are overexpressed in the diffuse gastric cancer subtype and are associated with tumor progression and peritoneal dissemination[23]. The analysis of proteins contained in exosomes secreted by cancer cells revealed that insulin receptor signaling affects tumor cell invasion as it modulates E-cadherin glycosylation thus increasing the expression of mesenchymal markers[24]. More recently, the secreted Interleukin-1 receptor accessory protein has been defined as relevant as its expression is significantly increased in successive stages of gastric adenocarcinoma[25]. Immune infiltration analysis in the tumor microenvironment has identified two subtypes of cancer: immunological hot and cold; these different phenotypes are relevant to the therapeutic response to immune checkpoint inhibitors[26]. This type of analysis in gastric cancer demonstrated that the overexpression of the tight junction protein claudin 3 in the “cold” tumors is associated with inhibition of MHC-1 and CXCL9 expression and poor infiltration of CD8+ T cells[27].

Microbiomics

Despite the progress in the identification of specific factors contributing to gastric carcinogenesis (genes, proteins, metabolic molecules, and pathways, sensitivity to immune effectors), there is ample evidence suggesting that the gut microbiome can foster epigenetic alterations and mutagenesis on the host genome and impact responses to cancer therapy, especially by influencing the response to immune checkpoints blockade[28]. The preservation of the spatial relationship between microbiota and the epithelial surface is essential to avoid harmful immune responses but it is regulated by functional oscillations in the metabolome patterns that determine the exposure of the epithelium to different bacterial species and their metabolites[29]. When commensal bacteria are disrupted the consequent dysbiosis can lead to impaired local, regional and systemic immune responses that incite a profound inflammatory state[30]. Niche-specific microbiota

alterations have been reported during the progression from gastritis to gastric cancer although *H. pylori* mainly affected the gastric corpus microbiota and not the gastric antrum. Similarly, gastric cancer-specific stomach peritumoral and tumoral microhabitats determine the composition and diversity of the gastric microbiota[31] which is composed mainly by *Firmicutes*, *Bacteriodetes*, *Actinobacteria*, *Fusobacteria,* and *Proteobacteria*. Interestingly a low gastric microbial dysbiosis and distinct dietary patterns-vegetable and seafood in males and high dairy consumption in females- reduce the gastric cancer risk[32]. The major bacterial metabolites associated with gastric cancer development are polyamines, N-nitroso compounds, and lactate which play a role in immune escape and suppressing the antitumor immunity[33]. Metatranscriptomic analysis of the gastric microbiota in human corpus premalignant tissue demonstrated *H. pylori* abundance and high expression of genes involved in pH regulation (urea and ureB), nickel availability (hpn and hpn2) and oxidative damage protection (katA, trxA, tsaA, fldA, and sodB)[34]. Nevertheless, recent evidence suggests that gastric dysbiosis imbalance after *H. pylori* eradication may be associated with gastric cancer development[35].

Can system vaccinology be considered as an omic?

Omics analysis has helped in the identification of dysregulated genes, epigenetic abnormalities, altered transcription mechanisms, affected cellular pathways, overexpressed or atypical proteins and/or receptors, and modified sugar-lipid-amino acids metabolites, associated with gastric carcinogenesis. All of them have been evaluated as possible biomarkers of early detection, tumor subtype, prognosis, and survival probability but an essential question remains can we produce long-term protection against the development of gastric cancer? Can all this information lead to a protective vaccine?

The integration of all the omics has favored the identification of potentially new antigens that have been used to generate a vaccine against several solid tumors[36]. The advent of high-throughput technologies coupled with systems biological methods, an approach to understanding the larger picture of tissues or cells, has enabled the characterization the identification of predictive signatures of vaccine response. The latter, known as systems vaccinology[37], coupled with cytometry analysis and its integration with omics information will certainly be applicable for vaccine development[38].

Perspectives

So far, the major advances in high-throughput omics methodologies have been the discovery of mechanisms involved in vaccine protection, immune memory, secondary effects, and mostly the development of more efficient antigens.

Overall a comprehensive overview of the reported associations between DNA variations showed that genetic variants significantly associated with the risk of gastric carcinoma were associated with cell signal transduction (IGFBP3, PLCE1, PPARG, and PRKAA1), cell adhesion (ABO, MUC1, THBS3, and TIMP2), cell apoptosis/proliferation (CASP8, MDM2, MTX1, TP53, and PSCA), cell metabolism (EPHX1, GSTP1, and PKLR), and immunity/inflammation (IL-1b, IL-8, IL-10, IL-17F, TGFβR2, TNF, TLR4, and PTGS2). These results suggest that the exploration for gastric cancer-specific genes is subject to genetic variations affecting different populations so its usefulness as diagnostic biomarkers could be considered as restricted.

So far, the most commonly clinically available biomarkers of gastric cancer include CEA, CA19-9, CA72-4, AFP, CA125, and HER2 but due to their poor specificity and sensitivity, their use as dependable tumor biomarkers is limited. The current evaluation of Fibroblast Growth Factor Receptor 2, E-cadherin, Akt, PDL1, MET, VEGFR2, TP53, and Claudin-6[39-44] have proved to be of more clinical value than the comprehensive genomic analysis for the early diagnosis and prognosis of gastric cancer[45]. We strongly believe that an evaluation of the possible abnormalities accompanying each of the different cell populations that constitute the gastric epithelial could help in the understanding of the vast differences observed in the histology and the omics of gastric cancer.

Conclusion

Although the conceptual pathway for gastric cancer vaccine development is clear and the use of bioinformatic analysis embraced in the omics concept has provided important tools to understand the biology of gastric cancer and the regulatory role that the tumor microenvironment exerts, we are a long distance to achieve our main goal, that is, to diagnose gastric cancer in the early stages based on detection of specific genes and to treat patients with specific pathways inhibitors and/or immune suppressors.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article. Furthermore, they have acknowledged and followed the recommendations as per the SAGER guidelines depending on the type and nature of the study.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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