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REVIEW: GASTRIC MALIGNANCIES – BUGS, PATHWAYS AND MOLECULAR PROGRAMS

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Abstract – Gastric cancer remains one of the most frequent and lethal cancers worldwide, as a consequence of aging and lack of screening programs. Moreover, the detailed mechanisms underlying gastric carcinogenesis have yet to be disclosed, which impairs accurate predictions of patient outcome and effective therapeutic strategies. In the last year, we have witnessed the emergence of new data that reinforce the complex interplay between microbes and other risk factors in the development of this disease. In particular, basic and clinical investigations have elucidated oncogenic mechanisms exerted by *Helicobacter pylori* and Epstein-Barr virus infection, involving the activation of pro-inflammatory signaling and acquisition of genomic instability. The gastric microbial community was also found to modify the stomach microenvironment, yielding distinct morphological and biochemical profiles that promote gastric carcinogenesis.
Herein, we selected relevant studies published from April 2021 to March 2022, and provided an overview of novel pathways and molecular programs underlying gastric cancer development and therapy resistance. Ultimately, we shall discuss how this knowledge may improve classification systems, and highlight potential molecules as therapeutic targets or as prognostic biomarkers.

Keywords: Gastric cancer, Microbial risk factors, Cancer signaling, Cancer therapy.

INTRODUCTION

Gastric cancer remains as one of the most frequent and deadliest malignancies worldwide. The last estimate of Globocan[1] ranked gastric cancer as the fifth most incident cancer, with more than 1 million new cases per year, and the fourth leading cause of cancer death[1]. Recent predictions indicate that global gastric cancer incidence will continue to decrease through 2030, with the exception of some countries such as Ecuador and Lithuania[2].

Gastric cancer develops as consequence of a long and complex interaction between microorganisms, environmental factors, and host genetic susceptibility. This disease exhibits distinct morphological and genetic profiles, which are reflected in distinct histological and molecular subtypes[3,4]. The heterogeneous nature of gastric cancer is indeed a major issue in diagnosis and selection of effective treatment strategies. Therefore, understanding the contribution of risk factors and decoding the molecular profile of gastric cancer may provide an unprecedented opportunity to improve diagnosis, treatment and management of cancer patients.

RISK FACTORS FOR GASTRIC CANCER

Helicobacter pylori Infection

H. pylori infection is the major risk factor for gastric cancer, but the oncogenic mechanisms triggered by the bacterium remain to be fully disclosed. An infection hallmark is the induction of inflammation that can activate several pathways, including the NF-kB signaling, which regulates innate and adaptive immune functions. Interestingly, Maubach et al[5] identified a double role of TRAF-interacting protein with FHA domain-containing protein A (TIFA) in the activation of both classical and alternative NF-kB signaling upon *H. pylori* infection. Classical NF-kB signaling was shown to be dependent on the interaction of TIFA with TNF receptor-associated factor 6 (TRAF6) allowing the binding of TGFβ-activated kinase 1. In contrast, an alternative pathway was activated after TIFA/TRAF2 interaction and led to displacement of the cellular inhibitor of apoptosis 1 (cIAP1) for proteosomal degradation[5].

Imai et al[6] described a new mechanism mediated by *H. pylori* CagA-positive strains, in which the oncoprotein CagA interacts with PAR1b to suppress BRCA1 phosphorylation and nuclear translocation in gastric cells. Consistent with its role in DNA repair mechanisms, BRCA loss-of-function increased DNA double-strand breaks and genomic instability, providing cells with a remarkable capacity to escape apoptosis[6].

In line with the current view of anti-*H. pylori* therapy as a major contributor to mitigate gastric cancer risk, Chiang et al[7] evaluated the benefits of *H. pylori* mass eradication initiated in 2004 in a high-risk population in the Matsu Islands (Taiwan). After six rounds of eradication and intensive screening that covered 85% of the population, a significant decrease in *H. pylori* prevalence and gastric cancer incidence was observed. Of note, this massive intervention had no significant impact on the incidence of other gastrointestinal cancers or on the rate of *H. pylori* antibiotic resistance[7]. The benefits of *H. pylori* eradication on individuals with precancerous lesions were also investigated by Piazuelo et al[8] using a chemoprevention approach. The authors concluded that after 20 years of anti-*H. pylori* treatment, the progression of premalignant lesions was delayed by 41%, and multi-focal atrophic gastritis without intestinal metaplasia reverted to non-atrophic gastritis[8].

The advantages of massive *H. pylori* eradication programs to prevent progression of precancerous lesions and decrease gastric cancer incidence are, however, debatable since bacterial resistance to antibiotics may compromise the benefits of *H. pylori* eradication. Mannion et al. showed that a significant number of *H. pylori* isolates (20%) acquire genetic mutations, which confer resistance to levofloxacin and to metronidazole[9]. In agreement with this data, Ziver-Sarp et al[10] detected a great number of strains showing resistance to clarithromycin (37%) and to levofloxacin (22%). Importantly, both studies showed an emergent number of strains containing resistance to both antibiotics tested and they recommend that multi-resistant strains should be controlled by constant screening.

Viral Infections

About 9% of gastric cancers are associated with Epstein-Barr virus (EBV), even though *H. pylori* infection can also be detected in some of these cases. A recent observational study comprised of 100 gastric cancer patients showed co-infection of EBV and *H. pylori* in 16% of the cases[11]. Consistent with this, a larger epidemiological study that included cancer patients and controls revealed a 3.3-fold increased risk for gastric cancer in the presence of co-infections[12]. Therefore, the investigation of the mechanisms underlying gastric cancer associated with co-infections has gained increasing interest. Fekadu et al[13] demonstrated in an *in vitro* model that *H. pylori* infection changes the expression of accessory EBV receptors, namely EphA2 and NMHC-IIA, endowing the virus with an increased infection efficiency. Likewise, Kashyap et al[14] showed that, alongside the expression of important virulence factors of EBV and *H. pylori*, co-infection increases the levels of host gankyrin, modulating several oncogenic pathways related with growth, proliferation, DNA response and migration of cells.

Gastric Microbiota

Evidence has emerged showing that the microbial community of the stomach plays a role in gastric carcinogenesis[15]. Taking advantage of gastric specimens, faecal samples or oral washes, several studies have demonstrated the presence of dysbiosis in gastric cancer cases versus controls[15]. Nevertheless,only a small number of studies has analyzed the microbiome in preneoplastic lesions. Wu et al[16] performed a case-control study on 89 patients with intestinal metaplasia and matched controls and analysed their respective microbiome by whole metagenome sequencing[16]. *Peptostreptococcus stomatis*, *Johnsonella ignava*, and *Filifactor alocis*, which are related to periodontal disease, as well as other known opportunistic pathogens such as *Neisseria elongata* and *Neisseria flavescens*, were identified in gastric intestinal metaplasia cases, supporting the hypothesis that members of the oral cavity are involved in gastric carcinogenesis[16].

By comparison of tumour and adjacent non-tumour tissues using 16S rRNA sequencing, Dai et al[17] verified that the abundance of *H. pylori* was higher in non-tumour tissues, while *Lactobacillus* and *Streptococcus* species were increased in tumour samples. Furthermore, the implementation of a metabolomics workflow revealed 150 metabolites able to differentiate tumour tissues from paired non-tumour tissues. Interestingly, *Helicobacter* and *Lactobacillus* abundances were correlated with particular metabolites of the amino acid class. Based on these findings, the authors suggest that tumour microbial metabolism yields metabolites that serve as energy sources (amino acids and its precursors) for neoplastic cells[17].

A differentiating approach was developed by Know et al[18] to evaluate histopathological alterations in the stomachs of germ-free mice transplanted with the microbiota of patients with premalignant lesions or with gastric cancer. The authors reported that microbial communities transplanted from patients with intestinal metaplasia and gastric cancer effectively colonised the stomachs of mice and induced major histological alterations, namely *foci* of intense inflammation accompanied by increased expression of proliferative and metaplastic markers. One year after inoculation, histological changes were even more apparent and mainly characterised by dysplastic areas[18].

Other Risk Factors

It is well established that host genetic susceptibility contributes to gastric cancer risk. Variants in genes implicated in the inflammatory response to *H. pylori* infection have been associated with increased risk of cancer, since its first description in 2000[19]. Nonetheless, other genes with oncogenic functions have also been proposed as susceptibility factors. Huang et al[20] investigated the relevance of polymorphisms in PIN1, a peptidylprolyl isomerase that binds to phosphoproteins and catalyses the cis-trans isomerization of proline peptidyl bonds, regulating several proteins involved in cancer initiation and progression. The promoter rs2233678 variant was inversely correlated with gastric cancer risk, whereas the rs2233679 variant conferred a mild increased susceptibility to gastric cancer[20]. In a large case-control study involving patients from Honduras, Miller *et al*. detected 94 variants in 54 genes and demonstrated that variants in CASP1, a key gene in inflammatory-induced cell death, and in TLR4, a sensing receptor for lipopolysaccharide of gram-negative bacteria, confer increased risk for gastric cancer[21]. An additional variant in the ODC1 gene, involved in the polyamine biosynthesis pathway, was identified and associated with a strong risk for gastric cancer even after adjustment for age, sex and CagA seropositivity[21].

Proton pump inhibitors (PPIs) used in heartburn, acid-related disorders, and in *H. pylori* eradication regimens have been described to induce hypergastrinaemia and stomach hyperplasia facilitating cancer development. Accordingly, two large population studies, one performed in United Kingdom[22] and the other in Korea[23], concluded that the use of PPIs significantly contributes to an increased gastric cancer risk and proposed that long-term PPIs should be used with caution, particularly in high-risk areas[22,23].

MOLECULAR CLASSIFICATION OF GASTRIC CANCER

Comprehensive molecular classification of gastric tumours became an essential tool to predict clinical outcome and assist treatment decision. In this sense, Chen et al[24] proposed a

molecular classification of three subtypes based on long-noncoding RNA (lncRNA) analysis. They unveiled that lncRNA subtype 3, which represents part of the intestinal type of gastric cancers, includes the cases with worse survival and with increased probability of developing distal metastasis, whereas the lncRNA subtype 1 represents the cases with a better survival rate[24]. This new classification system effectively discriminated between high-risk cases and worse survival, and suggested LINC01614 as a relevant prognosis biomarker[24]. Using an approach focused on genetic alterations, Wang *et al*. found mutations in the histone-lysine N-methyltransferase 2 (KMT2) in 10% of gastric cancer cases[25]. These cases displayed microsatellite instability, higher mutation rates in receptor tyrosine kinases, and increased PD-L1 positivity when compared with the wild-type KMT2 cases. Overall, this work indicates that patients carrying KMT2 mutations may benefit from immune checkpoint inhibitors, as well as drugs targeting DNA damage repair, MAPK-PI3K, metabolism, and cell cycle pathways[25]. An innovative strategy based on single-cell RNA sequencing was implemented to explore intra-tumoural heterogeneity[26]. Distinct gene expression profiles allowed the categorisation of five cellular subgroups. Among these, three subtypes showed typical features of the histological subtypes proposed in the Lauren's classification. Another subtype depicted intestinal-type gastric cancer with EBV-infection and was characterised by expression of immune-related genes. Interestingly, one subtype exhibited chief-cell markers and activated the Wnt/β-catenin signaling pathway, suggesting a possible differentiation of chief-cells into metaplastic cells[26].

CANCER SIGNALING AND MOLECULAR PROGRAMS

The past few years have witnessed the emergence of groundbreaking experimental approaches that unveiled exciting molecular mechanisms underlying gastric cancer aetiology. In particular, significant work has been produced on the interplay between the extracellular matrix (ECM) and cancer cells. Integrin binding to ECM proteins activates Ras-mitogen-activated protein kinase (MAPK/ERK) and phosphatidylinositol 3-kinase (PI3K/AKT) pathways, regulating cellular processes such as survival, differentiation, and invasion[27].

Consistent with this, a recent study revealed an ECM signature associated with gastric carcinogenesis[28]. By exploring a combined strategy of decellularisation techniques with high-throughput quantitative proteomics, the authors characterised gastric tumour, normal adjacent and normal distant mucosa, ultimately defining the matrisome composition of each tissue. This work revealed an ECM remodeling during transformation of gastric mucosa, encompassing alterations in the abundance of 24 components, mainly basement membrane proteins[28]. Functional analysis of the data suggested that ECM changes lead to activation of ECM receptors and cellular processes involved in angiogenesis and cell-extrinsic metabolic regulation[28]. Noteworthy, RNA-seq from samples available at The Cancer Genome Atlas (TCGA) emphasised the importance of COL1A2, LOX and LTBP2 as potential biomarkers of disease progression[28].

The role of ECM receptors in gastric cancer was dissected by Figueiredo et al[29] in a pioneer study taking advantage of an ECM microarray incorporating 36 combinations of ECM proteins. This screening method pinpointed Integrin β1 as a key player in the initial steps of the invasive process mediated by E-cadherin dysfunction, promoting cell-matrix adhesion, cell-cell loosening and motile properties. The clinical significance of these findings was subsequently validated in transcriptomic data of 262 gastric cancers. Cases presenting low E-cadherin expression levels exhibited high levels of integrin β1 and were associated with increased tumour grade and poor overall patient survival[29].

The deleterious effects induced by loss of E-cadherin were further investigated in the context of Hereditary Diffuse Gastric Cancer (HDGC). HDGC is a rare highly invasive cancer syndrome caused by pathogenic germline variants in *CDH1* gene, which encodes E-cadherin[30]. However, the molecular mechanisms underlying neoplastic transformation induced by E-cadherin loss remain largely unknown. To address differentiation trajectories that take place in the early steps of tumourigenesis, Dixon et al31 used mouse tissue-derived organoids and single-cell RNA sequencing. Increased expression of *Krt19* and *Krt7*, encoding the differentiation markers cytokeratin 19 and cytokeratin 7, was found in E-cadherin negative cell populations,

in comparison with wild-type counterparts[31]. Furthermore, a strong cytoplasmic staining of cytokeratin 7 was detected in invasive signet ring cells (SRCs) from carriers of pathogenic germline variants, supporting its relevance in early pathogenesis of HDGC and its potential as a clinical biomarker[31]. Corroborating a dynamic regulation of differentiation genes in gastric cancer, *Krt17* was found to be decreased in diffuse-type gastric carcinoma, when compared with the intestinal-type[32]. Interestingly, lower levels of *Krt17* induce cytoskeletal reorganisation and activation of the YAP signaling pathway, contributing to epithelial to mesenchymal transition and to the metastatic potential of gastric cancer cells[33].

Altogether, these reports provide important insights on gastric cancer biology, and highlight a possible synergy between the ECM and differentiation programmes during gastric cancer development.

GASTRIC CANCER TARGETED THERAPY

Programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1) on cytotoxic T lymphocytes are known to interact, prompting tumours to avoid immune surveillance. Therefore, the blockage of this pathway with antibodies targeting PD-1 has been recently explored with success in different cancer types. Despite the fact that around 40% of gastric cancers display PD-L1 expression, only a small fraction of them respond to immunotherapy[33].

The mechanisms of tumour resistance to inhibitors of PD-1 were addressed by Kim et al. in a mouse model of gastric cancer[34]. The researchers showed that the use of chemotherapeutic agents, namely 5-fluorouracil and oxaliplatin, decreased the number of myeloid-derived suppressor cells and increased the effects of anti-PD-1 therapy[34]. Nevertheless, these drugs also promoted the expression of PD-L1 in tumour cells, which led to a gain in tumourigenesis in the presence of oncogenic agents such as *H. pylori* or N-methyl-N-nitrosourea. They concluded that the location and timing of PD-L1 expression is critical for gastric tumour progression and should be considered in therapeutic regimens[34].

The therapeutic response is also affected by the tumour microenvironment. Hence, Sundar *et al*. combined the analysis of epigenetic alterations in promoter regions with single-cell RNA-seq to delineate the impact of the tumour microenvironment on immune escape and immunotherapy resistance[35]. Gastric cancer cases with high alternate promoter burden have lower T-cell infiltration and poorer progression-free survival than those cases with low alternate promoter burden. Importantly, resistance to immune checkpoint inhibitors was confirmed in patients presenting high alternate promoter burden[35].

In contrast to previous reports that focused on the relationship between the efficacy of cancer immunotherapy and intestinal microbiota, Oster *et al.* provided the first evidence that *H. pylori* infection also influences this response. Accordingly, *H. pylori* serology was recommended to personalise the treatment with immune checkpoint inhibitors[36].

Regarding conventional chemotherapeutic modalities, an important step was taken to disclose the mechanisms responsible for the acquisition of trastuzumab resistance in ErbB2-positive gastric cancer. Duarte et al[37] performed a glycomic and glycoproteomic profiling of ErbB2's ectodomain from gastric cancer cells and identified the ST6Gal1 sialyltransferase as a specific regulator of N-glycosylation occurring at the binding domain of trastuzumab. Of note, depletion of ST6Gal1 expression led to increased ErbB2 half-life and stability at the plasma membrane, improving the therapeutic performance of trastuzumab while decreasing the activation of ErbB2 and EGFR downstream pathways37. Those results suggest that a characterisation of ErbB2 glycoforms may be useful for patient stratification and prediction of therapeutic response to trastuzumab.

CONCLUSIONS

In the past year, compelling advances have contributed to our understanding of the risk factors and consequent signaling cascades underlying gastric cancer development. Experimental and clinical evidence have emerged exposing specific effects of *H. pylori*, EBV infection, and

microbial communities in gastric tissue. Moreover, the ECM, differentiation programmes, and immune response have arisen as key regulators of a pro-tumourigenic phenotype impacting gastric cancer progression and prognosis. This unlocks new avenues of research focusing on the interplay between the microbiome and the tumor microenvironment. A fundamental question to be addressed is how bacteria modulate both the cellular and non-cellular components of the tumor ecosystem, namely immune cells and the ECM, promoting gastric cancer development. We foresee this knowledge could be translated into tailored strategies for effective gastric cancer prevention and treatment.

Conflict of Interest

The authors declare no conflict of interest.

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