Chapter

Epstein-Barr Virus-Associated Gastric Cancer: Old Entity with New Relevance

Hugo Manuel Lopes de Sousa, Joana Patrícia Costa Ribeiro and Mafalda Basílio Timóteo

Abstract

Gastric cancer (GC) represents a major public health issue worldwide, being the fifth most common cancer and one of the leading causes of death by cancer. In 2014, The Cancer Genome Atlas (TCGA) established that tumors positive for Epstein-Barr virus (EBV) are considered a specific subtype of GC (EBVaGC). Several meta-analyses have shown that EBVaGC represents almost 10% of all gastric cancer worldwide, with small differences in the geographic distribution. This tumor subtype has a high potential of being clinically relevant and studies have shown that it has specific features, a better prognosis, and increased overall survival. In this review, we summarize some of the most frequent aspects of EBVaGC, including the specific features of this GC subtype, data regarding the potential steps of EBVaGC carcinogenesis, and perspectives on treatment opportunities.

Keywords: Epstein-Barr virus, gastric cancer, carcinogenesis, p53, PDL-1, immunotherapy

1. Introduction (Gastric cancer)

1.1 Epidemiology

Gastric cancer (GC) represents a major public health issue worldwide, being the fifth most common cancer and one of the leading causes of death by cancer [1, 2]. GC affects more than 1,000,000 people per year and leads to approximately 783,000 deaths each year, corresponding to 5.7% of new cases and 8.2% of all cancer related deaths (**Figure 1**). Worldwide, GC incidence has a distinct geographic distribution pattern [3, 4] (**Figure 2**). The highest incidence rates are registered in Eastern Asia and Central/Eastern Europe, while Northern America and Africa have the lowest incidence rates [1, 5].

There seems to exist some ethnic/racial disparities in the distribution of GC [6, 7]; nevertheless studies showed that this may be the explained by the different expositions to GC risk factors such as dietary, salt intake, and *Helicobacter pylori* infection [5, 8]. Furthermore, despite the worrying high mortality associated with GC, the incidence of GC globally has been declining since 1990. This trend is mostly due to the falling rates of non-cardia GC, which is explained by the improvement of hygienic conditions and early detection of cancer strategies [9].

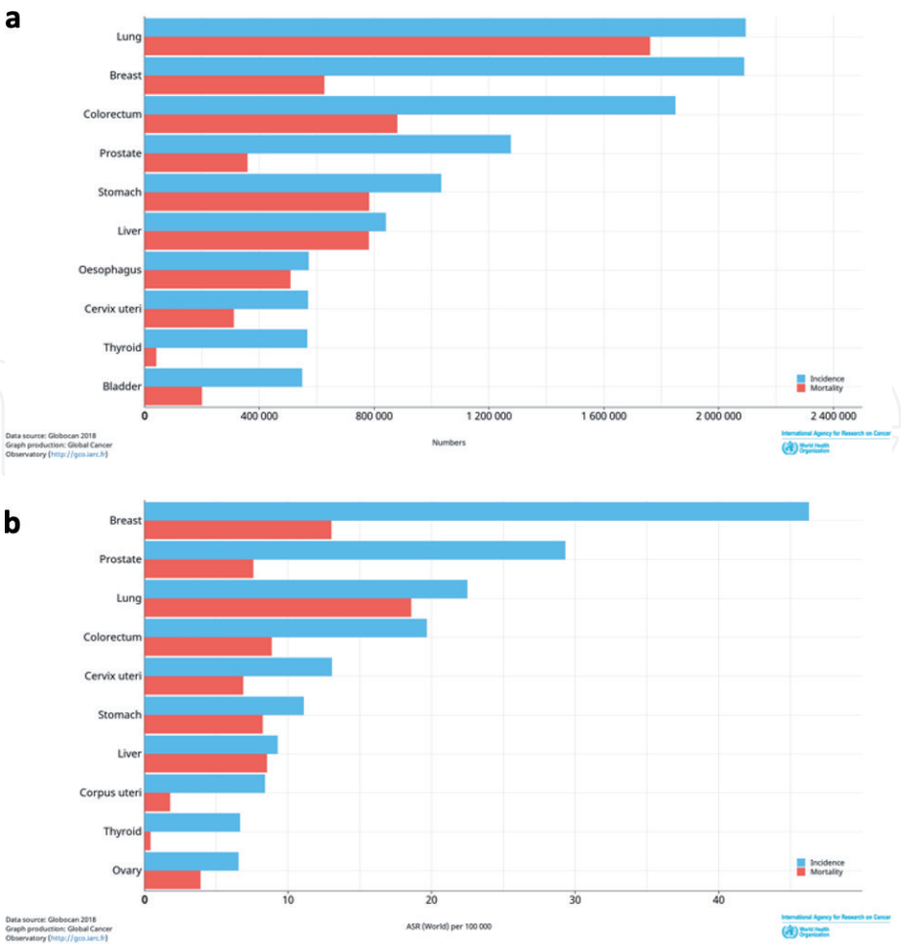

Figure 1.
Estimated number (a) of incident cases and deaths of gastric cancer worldwide and (b) age-standardized rates (GLOBOCAN 2018).

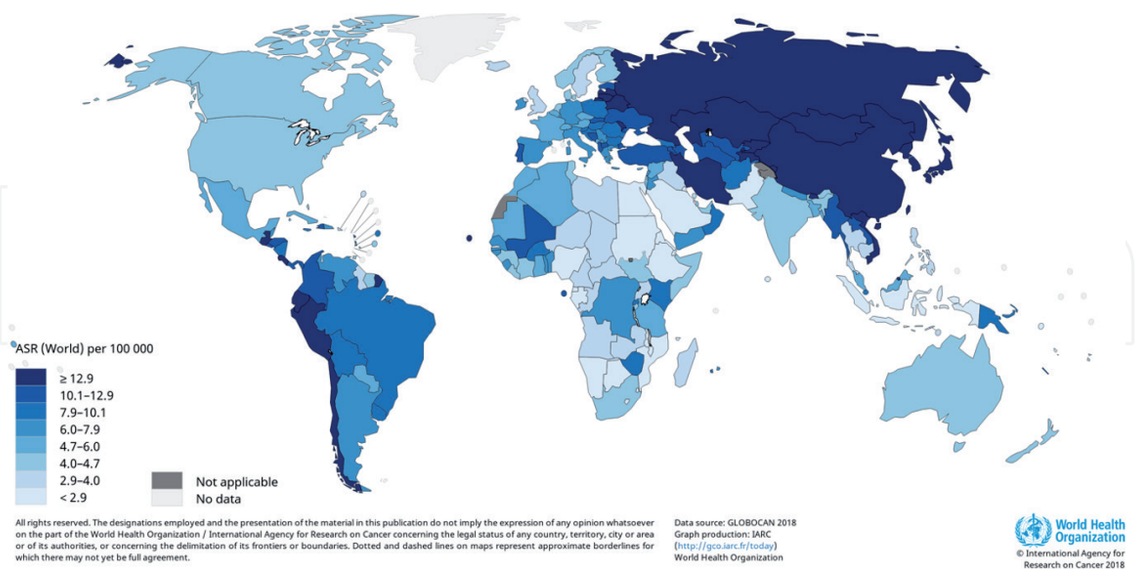

Figure 2.
Gastric cancer age-standardized rate incidence worldwide (GLOBOCAN 2018).

1.2 Classification

GC classification has been changing according to its anatomical, histological, or molecular features without a consensus regarding the best system combining prognosis and high practicality in clinical diagnosis [10–16]. For many years,

the anatomical classification was used to distinguish cardia and non-cardia GC, which have distinct etiological and epidemiological characteristics [3, 6, 11]. Two classification systems that have been used for diagnosis and treatment decisions are the *Lauren classification* and *World Health Organization* (WHO) *classification*; nevertheless, the clinical impact is still limited [10, 17].

The *Lauren classification* divides gastric adenocarcinomas into diffuse, intestinal, and intermediate type, which combines cancers with uncommon histology [10, 18, 19]. There are multiple evidences indicating that the two principal subtypes may have distinct tumor development pathways [10, 18]. The intestinal type presents specific characteristics such as well/moderate differentiation of cells, loss of E-cadherin expression and is associated with H. *p*ylori infection [7, 20]. The carcinogenesis model of this subtype is characterized by a progressive model characterized by chronic gastritis and gastric mucosa metaplasia [10]. The diffuse type is characterized by poorly differentiated cells with cellular atypia and numerous mitotic figures and poorly cohesive structure, and therefore it is more aggressive and with worse prognosis [11, 18, 19]. The WHO classification divides GC according to the histological features of each subtype: papillary, tubular and mucinous adenocarcinomas, poorly cohesive (including signet-ring cell carcinomas), mixed carcinomas (with two or more components), and uncommon variants [21, 22].

In 2014, *The Cancer Genome Atlas* (TCGA) consortium group proposed a classification of gastric adenocarcinomas into four distinct subtypes based on their molecular features, which may have a higher clinical impact in treatment prediction and prognosis: (1) microsatellite unstable tumors (MSI), (2) genomically stable tumors (GS), (3) tumors with chromosomal instability (CIN), and (4) tumors positive for Epstein-Barr virus (EBVaGC) [17, 22, 23] (**Figure 3**). Later in 2018, Hinoue et al. described another subtype of GC, characterized by hypermutated status with single-nucleotide variants (hypermutated-SNV, HM-SNV) [24, 25]. This system seems to have a higher clinical impact in treatment prediction and prognosis when compared with previous classification systems [26, 27]. Later, the *Asian Cancer Research Group (ACRG)* has proposed a new classification according to patterns of molecular alterations, disease progression, and prognosis: (1) high microsatellite instable (MSI-high) tumors, (2) microsatellite stable with epithelial-to-mesenchymal transition (MSS/EMT) phenotype tumors, (3) microsatellite stable with *TP53*

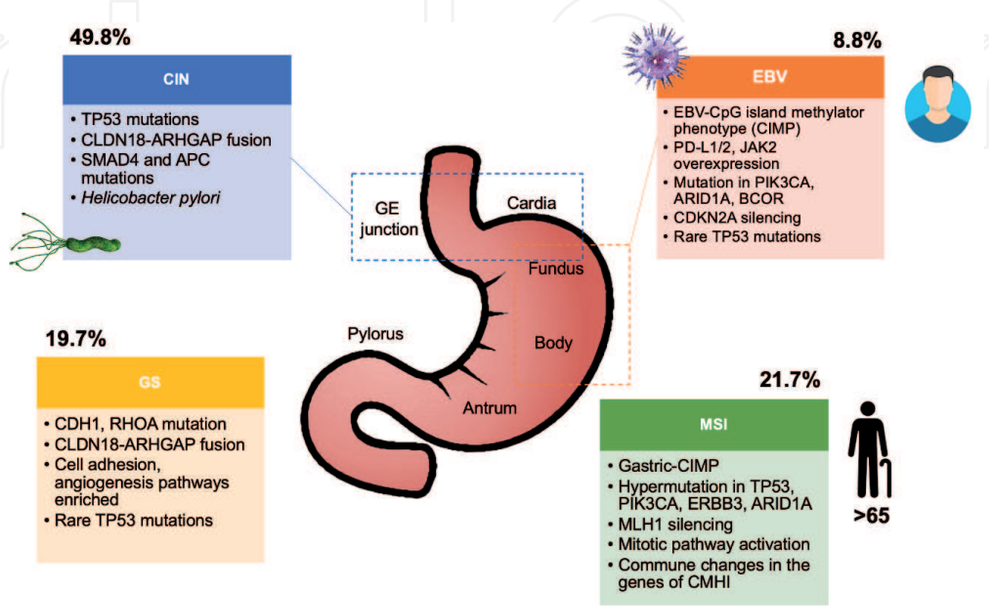

Figure 3.
Essential features of gastric cancer subtypes according to the Cancer genome atlas research network.

intact (MSS/TP53+), and (4) microsatellite stable with *TP53* loss (MSS/TP53-) [16, 28]. It is possible to obtain a partial correspondence between the TCGA and ACRG classifications, although EBV is not specifically included in the ACRG classification, EBV infection was frequently observed in the MSS/TP53+ subtype [29].

Additional subtypes of GC have been described based on the TCGA and ACRG classifications and specific analysis of different genetic features [29–31]. Nevertheless, independently of the classification system, EBV-positive GCs are considered to be of better prognosis [26, 27].

1.3 Gastric carcinogenesis and risk factors

Gastric cells' malignant transformation is a multistep process in which risk factors, genetic or epigenetic alterations can be observed [32, 33]. The carcinogenesis model for the other GC subtypes still remains a challenge for scientists due to the different histological subtypes [18, 34, 35]. The most accepted hypothesis of gastric carcinogenesis has been described for the intestinal subtype according to the *Lauren's* classification, and it is characterized by a cascade of progression from normal gastric epithelium through chronic gastritis (CG), chronic atrophic gastritis (CAG), and intestinal metaplasia (IM), ultimately leading to dysplasia and carcinoma [36, 37].

There are common risk factors for GC that can be subdivided into modifiable and non-modifiable. The non-modifiable factors include age, male gender and familiar history, and inherited syndromes, such as familial adenomatous polyposis (FAP) and Peutz-Jeghers syndrome (PJS), hereditary diffuse gastric cancer (HDGC) or Lynch syndrome [7, 38–40]. Host genetic polymorphisms have also been described to contribute to an increased risk pattern for GC development [41].

The modifiable risk factors can be divided in two major groups: dietary/lifestyle influences and infectious agents [39]. Dietary and lifestyle risk factors for GC include salt and salted preserved food, fruits and vegetables, tobacco, alcohol and body mass index (BMI), and physical activity [38, 42]. Data suggest that high salt consumption is responsible for a two-fold increase in the risk of GC development when compared to low salt intake, mainly because it induces early atrophic gastritis [39, 43]. Conversely, consumption of fresh fruits and vegetables, with vitamins C and E, carotenoids, and selenium has been associated with a decreased risk of GC in around 20-30% [7, 20]. As in other types of cancer, studies suggest that tobacco smoking is responsible for a 1.5-fold increased relative risk of developing GC [7]. Despite no explicit association, alcohol consumption is also associated with increase in risk of gastric cancer [7, 44]. A meta-analysis study has shown that high body mass index (BMI) (>25) increases the risk to develop non-cardia gastric cancer, which is 1.4-fold for overweight and two-fold in obese individuals. Conversely, regular physical activity seems to be associated with lower risk of GC [39, 45].

H. pylori infection affects around 50% of world population and has been classified by World Health organization (WHO) as a class I carcinogen being responsible for a two-fold increase in the risk of developing non-cardia gastric adenocarcinoma [7, 46, 47]. H. pylori contributes to gastric carcinogenesis by inducing chronic gastritis that over time may progress to severe atrophic gastritis, which in turn can develop to cancer [20, 44]. Other risk factors have been described as contributing to increase the risk of persistent H. pylori infection and therefore to GC development [48]. The Epstein-Barr virus [49] is another infectious agent accepted as associated with gastric carcinogenesis, however, the mechanism of action in gastric carcinogenesis is still unknown [44, 50, 51].

2. EBV-associated gastric cancer

2.1 Historical background

Epstein-Barr virus [49] is linear, double-stranded DNA virus member of herpesviridae family, with a high prevalence worldwide (>90% of adults) [52]. EBV is recognized for establishing a latent infection with frequent reactivations and has been associated with the development of multiple diseases from infectious mononucleosis to different cancers [52–55]. EBV was the first virus to be recognized as the etiological cause of a human cancer, and since 1997, it is included by the *International Agency for Research on Cancer* in the Group-I carcinogen risk factors [56–58]. Indeed, EBV has been associated with several human tumors, including Burkitt's lymphoma, Hodgkin's disease, B cell lymphomas, and also some epithelial neoplasms such as nasopharyngeal carcinoma (NPC) or more recently GC [52, 59, 60].

The association between EBV and gastric carcinoma was first described in 1990, when Burke et al. used a polymerase chain reaction (PCR) technique to detect EBV in lymphoepithelioma-like gastric carcinomas, characterized by the presence of cells morphologically similar to the undifferentiated nasopharyngeal lymphoepithelioma [61]. Later, Shibata and colleagues have demonstrated by in situ hybridization that EBV infection was present in gastric carcinoma cells resembling lymphoepithelioma but not in reactive lymphoid infiltrate or normal mucosa [62]. Additionally, 1 year later, EBV infection was also detected in cases of typical gastric adenocarcinoma [62]. Over the past 30 years, GC has been consistently associated with EBV infection [51, 61, 63, 64] and EBV-associated gastric carcinoma (EBVaGC) which is now recognized as one specific subtype of GC [51, 65, 66].

2.2 Epidemiology and clinicopathological characteristics

Several meta-analyses have been attempting to summarize the association of EBV with CG development, showing that EBVaGC may represent almost 10% of all gastric cancer worldwide [66–74]. A recent meta-analysis with data from over 20,000 cancer patients within 26 different countries has shown that EBVaGC prevalence ranges from 1.69 to 43.75%, with a pooled prevalence of 8.77% (95% CI: 7.73–9.92%) and a pooled odds ratio (OR) of 18.56 (95% CI: 15.68–21.97) for studies with matched pairs and 3.31 (95% CI: 0.95–11.54) for studies with non-matched pairs design [72].

In contrast to others EBV-associated malignancies, EBVaGC is a non-endemic endemic disease distributed throughout the world. Data analysis showed no significant variation within the different world regions (8.21% in Europe, 8.38% in Asia, 9.51% in America, and 11.9% in Africa); indeed, it is possible to observe similar data in countries from Europe, including Portugal (8,4%) [75], Netherlands (7.8%) [67], and Denmark (7.6%) [76], and also from Asiatic countries such as South Korea (7.8%) [77] and Japan (8.0%) [78]. Nevertheless, it is still possible to observe differences among countries, and the differential expositions to risk factors are proposed as the possible explanation for the variation of EBVaGC prevalence [68, 69]. Indeed, some studies suggest that EBVaGC prevalence might be inversely correlated with the background incidence of GC [70].

EBVaGC seems to be more prevalent in younger patients and in males than in females (almost two-fold more prevalent), which has been a consistent association found in several, suggesting a potential association with lifestyle or hormonal factors [68, 69, 72, 79–84] (**Figure 4**). In addition, EBVaGC seems to be more frequently found in the proximal stomach and has a moderate to poor degree of differentiation

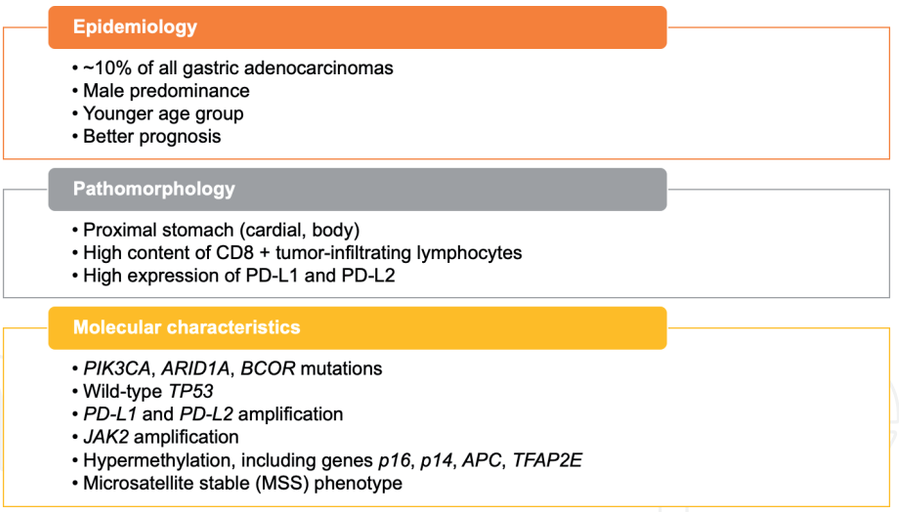

Figure 4.
Features of Epstein-Barr virus-associated gastric carcinomas.

[17, 58, 69, 72, 81, 82, 85–87]. These features are being suggested as potentially impacting the overall survival and recurrence of EBVaGC [26, 66, 80, 88], and a study with 4599 patients (pooled analysis) showed that EBVaGC has increased the overall survival and this is still a controversial topic [80]. One study evaluated the clinical significance of the different molecular subtypes of gastric cancer and concluded that EBVaGC, independently of the classification system, is of better prognosis [26, 27].

EBV association with gastric cancer was first described in lymphoepithelioma-like carcinoma also known as carcinoma with lymphoid stroma (GCLS) or medullary carcinomas [61, 88–90]. Recently, it has been described that there are three histological subtypes of EBVaGC based on the host cellular immune response status: the carcinomas with lymphoid stroma (GCLS), the carcinoma with Crohn's disease-like lymphoid reaction (CLR), and the conventional-type adenocarcinoma (CA) [91].GCLS is a rare histological subtype of gastric cancer, representing about 1-4% of all gastric cancers, of which literature shows that more than 90% of these cases are EBV-positive and characterized by poorly differentiated nests of neoplastic epithelial cells intermingled with a dense lymphoid proliferation [61, 63, 66, 69, 90, 92–94]. Nevertheless, literature has been focusing on the characterization of non-GCLs EBV-positive gastric cancers. This association remains controversial, and while several studies demonstrated a strong EBV association with a diffuse subtype [68, 95], others have reported a similar prevalence between intestinal and diffuse subtypes [67, 88, 96, 97]. Indeed, one meta-analysis has shown association with a diffuse subtype [66, 68], while two other meta-analyses did not find any association within histological subtypes [69, 74].

2.3 Diagnosis

The identification of EBVaGC has been performed by the identification of EBV transcripts in gastric tissues using in situ hybridization (ISH) by detecting EBV-encoded small RNAs (EBERs), which are highly expressed in latently EBV-infected cells [66, 91]. EBV-associated tumors are defined as monoclonal proliferations of carcinoma cells with latent EBV infection, and studies have confirmed that every cell from the cancer clone carries the clonal virus genome, suggesting that the virus was acquired before the transformation, even though it seems that it is not detected in precursor lesions [70, 86, 98].

The detection of EBV by PCR-based methods has been controversial since it frequently provides false positive results due to the presence of EBV-positive

lymphocytes in the surrounding tissue, ignoring that it might be absent in the tumor epithelial cells [66]. Therefore, EBER-ISH is considered the gold-standard method, and a positive EBV-associated case should be considered only if in the presence of EBERs in tumor cells and in its absence in the normal surrounding tissue [99].

2.4 Carcinogenesis mechanism

During the past decade, several authors have been discussing the mechanism of EBV carcinogenesis in GC [68, 100]. EBV is known to enter cells in oropharyngeal lymphoid tissue by the recognition/interaction with CR2/CD21 on the surface of B-lymphocytes that interact with EBV envelope glycoprotein gp350 [101, 102]. How and when EBV gets into gastric epithelial cells remains unclear, and it has been suggested that it can be either by cell-to-cell with B-lymphocytes recruited in inflammatory processes of gastric mucosa or through direct entry into the gastric epithelia [103]. This mechanism is not well understood and further studies should be made to establish if the recruitment of EBV-infected lymphoid cells might be the explanation for the infection and subsequent transformation of gastric epithelium.

Overall, literature suggests that EBV participates on gastric carcinogenesis by both direct and indirect mechanisms: infecting epithelial cells and establishing a latent program in which a restrict profile of latent proteins/transcripts are expressed; and/or promoting a chronic inflammatory response contributing to tissue damage and cancer progression [104, 105].

Previous studies regarding the detection of EBV in premalignant lesions of gastric cancer are extremely controversial [106–109] and the majority report its presence mainly in dysplasia and atrophic gastritis adjacent to tumors [87, 105–114]. A recent cross-sectional study from the North Region of Portugal showed no evidence of EBV infection in both dysplasia and early gastric carcinomas [75]. The absence of EBER transcripts in superficial gastric neoplastic lesions may suggest that EBV infection is a late event in gastric carcinogenesis [75]. Hence, it is still important to clarify the moment of EBV infection in gastric cells and if it acts as the initiator of carcinogenesis or as a promoter after prior modifications of gastric cells.

EBVaGCs are EBV-associated epithelial malignancies and therefore the mechanism of viral carcinogenesis might be similar to the observed in NPC. Two *in vitro* studies demonstrated that nasopharyngeal cells need to have some genetic change prior to be susceptible of EBV transformation [115, 116]. In fact, preexisting genetic events, mainly cyclin D1 overexpression and p16 mutations, seem to support the establishment of stable EBV infection and transformation in NPC epithelium [115, 116]. A recent publication suggests that EBV coordinates with somatic gene mutations in order to induce the carcinogenesis process in gastric epithelial cells [117]. This mechanism suggests that high-frequency mutations, such as in PIK3CA and ARID1A, are essential for the transformation of normal gastric cells into susceptible cells, which are more likely to be infected and transformed by EBV [117]. In addition, after infection, amplification of PD-L1 and PD-L2 are thought to increase the progression and immune evasion of transformed cells [117].

Some studies have been suggesting a possible interaction between H. pylori and EBV in gastric cancer development. Minoura-Etoh et al. observed a possible antagonism effect between H. pylori and EBV, showing that reactive products from H. pylori seem to induce EBV reactivation from latently in infected gastric epithelial cells, which would avoid the EBV transformation of gastric cells in the same areas of H. pylori colonization [118]. H. pylori seems to preferentially colonize the antral region, while EBV is more frequently found in the upper third and middle of stomach, suggesting a possible antagonism of EBV and H. pylori in gastric mucosa

[119–121]. By contrast, two other studies have suggested that H. pylori may contribute for EBV-associated gastric carcinogenesis by causing gastritis that perhaps might recruit EBV-carrying lymphocytes to the stomach wall, where the virus could be induced to replicate and infect gastric epithelial cells [122, 123]. Moreover, the gastric inflammation may also promote a cytokine-rich microenvironment, supporting a clonal growth of EBV-infected epithelial cells [110].

EBV establishes a latent infection allowing it to be maintained inside cells and to use the host machinery to express their own genes, regulating the cell behavior and escaping the immune system recognition [124, 125]. EBV latency is characterized by the expression of different viral proteins such as EBV nuclear antigens (EBNAs 1, 2, 3A, 3B, 3C, and EBNA-LP), EBV-encoded small RNAs (EBERs) 1 and 2, latent membrane protein (LMP 1, 2A, and 2B), and microRNAs from BamHI-A rightward transcripts, known as BARTs [58]. Depending on the infected cell type and differentiation status, different proteins are expressed, originating different latency profiles [124–126] (**Table 1**). Literature refers that the majority of EBVaGC cases show a latency II-like pattern (44%), defined by expression of EBNA1, EBERs, BARF1, and LMP2A genes, and latency I (42.9%) restricted to EBNA1, EBERs, and BARTs expression [75, 127]. The fact that different latency states seem to be associated to different malignancies explains the different mechanism of carcinogenesis on which EBV is involved and is thought to be important for EBVaGC characterization [124–126].

The function of the different EBV latent proteins has been widely studied. Each protein seems to have a significant role for the EBV cell cycle and transformation: EBNA1, expressed in every single infected cell, acts as a transcription factor responsible for the episomal maintenance, DNA replication, and indirectly to cell transformation [56, 65, 128, 129]; EBNA2 is early expressed in recently infected B cells, playing a crucial role in these cells' immortalization through the transcription of both viral and host genes [56, 130]; the EBNA3 protein family activates the transcription of cellular and viral genes, leading to the disruption of cell cycle checkpoints on different levels [56, 130]; LMP1, the major EBV oncogene, is essential for B-lymphocytes transformations, induction of apoptotic genes, epithelial cells transformations, and invasiveness and avoids cells apoptosis by different pathways [56, 125, 129, 131]; LMP2 essentially avoids the activation of the EBV lytic cycle in B-lymphocytes and modulates epithelial cell growth [56, 125, 129]; and EBER's role is not yet well understood but is thought to contribute to B cell transformation, acting as signaling and transcription factor regulators [56, 130]. EBV also encodes around

	EBNA1	EBNA2	EBNA3	LMP1	LMP2	EBERs	Malignancies
Latency I	x					x	Burkitt's lymphoma and gastric cancer
Latency II	x			x	x	x	Nasopharyngeal carcinoma, Hodgkin lymphoma, and T-cell non-Hodgkin lymphoma
Latency II-like	x				x	x	Gastric cancer
Latency III	x	x	x	x	x	x	Post-transplant lymphoma and AIDS-associated lymphoma

Table 1.
EBV-associated diseases' latency profiles.

40 miRNAs, which are known to bind and possibly participate in the regulation of hundreds of cellular and viral transcripts, some of them being involved in cell survival [56, 130]. These miRNAs can be referred as BHRF1 and BARTs, depending on its localization on the viral genome [130]. BHRF1 role is not very understood yet but some results suggest that BHRF1 miRNAs and proteins cooperate to control cell cycle initiation and apoptosis during primary infection [130]. Regarding BARTs, they are described as contributing to EBV-induced carcinogenesis by downregulating host genes, such as tumor suppressors and pro-apoptotic genes, including several cell growth and cell cycle-related [129, 130]. Nevertheless, is still important to clarify the coordination of virus and host cell in gastric cancer carcinogenesis.

2.5 Molecular features of EBVaGC

EBVaGC has some distinctive features in terms of genome alterations [17, 66, 132] (**Figure 4**). EBVaGC has been reported to have the most extensive CpG island methylation (human and viral genomes) than in any other tumor. This is described as EBV-CIMP (CpG island methylator phenotype) and includes genes related to cell cycle regulation (p14ARF, p15, p16INK4A, and p73), DNA repair (hMLH1, MGMT and GSTP1), cell adhesion and metastases (CDH1, TIMP1, and TIMP3), apoptosis (DAPK and bcl-2), and signal transduction (APC, PTEN, and RASSF1A) [17, 133–135].

EBVaGC is characterized by mutations in the PIK3CA gene and amplification of 9p24.1 locus containing JAK2, CD274, PDCD1LG2, and ERBB2 which contribute to altered proliferation, deregulation of apoptosis, and immune suppression and evasion [17, 136]. PIK3CA gene, which encodes phosphatidyl inositol-3-kinase (PIK3), has been consistently shown to be mutated in EBVaGC [17]. This protein is an important component of PI3K/Akt/mTOR signaling pathway and regulates several cellular processes such as apoptosis escape, cell growth, and proliferation [137]. Mutations in *PIK3CA* are common in several tumors, nevertheless in EBVaGC, about 80% are non-silent mutations and the vast majority are not located in the hot-spot sites but are dispersed in the gene sequence [17, 137]. EBVaGC has also been described as having mutations in other genes such as *ARID1A* and *BCOR* [17, 66, 132]. Interesting, *TP53* mutations that occur in the majority of gastric tumors are rare in EBVaGC, nevertheless a study has shown that these tumors seem to present a lower level of *TP53* mRNA and a higher level of p53 protein when compared with EBV-negative cancers, which increases the interest in studying the p53 pathway regulation [17, 138].

EBVaGC has also been described to have higher levels of programmed death ligands 1 and 2 (PD-L1/2) enriched with CD8 + tumor-infiltrating lymphocytes (TILs) and with high expression of immunogenic pathways [25, 139, 140]. Indeed, this is considered a highly immunogenic tumor with a great potential for immunotherapy [24].

2.6 Treatment options for EBVaGC

The unique molecular features of EBVaGC have gained interest in the past years, especially for the potential impact of targeted drugs since preclinical data have shown that EBVaGC is resistant to current chemotherapy [141].

PD-L1 overexpression has been consistently considered a marker for EBVaGC and MSI-high GC cases [142, 143]. Several PD-1 targeted drugs available on the market are being studied for its use in several cancers, including GC [141]. Pembrolizumab, a PD-1 antibody, was the first to be approved by the Food and Drug Administration (FDA) for use in recurrent MSI-high GC after a good rate response in several clinical trials (NCT03257163, NCT02589496) [142, 144–146]. Pembrolizumab has also been used for the treatment of EBV-positive T cell

lymphomas [147] and trials with EBVaGC are showing promising results [142]. Several clinical trials that include EBVaGC are testing other PD-1 target drugs, such as nivolumab (NCT02951091) or avelumab (NCT01772004), or by using CRISPR-Cas9-mediated PD-1 knockout EBV cytotoxic T cells (NCT03044743) [148–150]. Despite this, there are some controversial points regarding PD-L1 standardization and cutoffs, and the results from these studies point for an important role as a therapeutical target for GC, particularly in those with MSI-high or EBV. Indeed, EBV is now considered a biomarker for GC and the clear identification of EBVaGC in clinical series will contribute for the implement of better treatment strategies.

Literature shows that EBaGC has frequent *PIK3CA* mutations [17] and is thought to impact negatively the outcome of disease; nevertheless, the impact on the evolution of these cancers is still to understood [151–153]. PI3K/AKT/mTOR pathway inhibitors have been used as new therapeutical options in cancer, especially mTOR inhibitors such as everolimus, which are used in the phase III GRANITE-1 study (NCT00879333) for advanced GC with potential interest. More recently, PI3K inhibitors such as buparlisib (BKM120) have been tested for use in solid tumors [154, 155], and alpelisib has been tested for use as a potential therapeutical agent for gastric cancer [156]. There are a lot of PI3K/AKT/mTOR pathway inhibitors being used in GC clinical trials, and despite not being directed to EBVGC, a potential impact in this specific subgroup is expected.

Another important feature with potential therapeutical interest is the epigenetic changes of EBVaGC. It is known that epigenetic changes are reversible and therefore many de-methylating agents are been studied in cancer treatment. A few studies have reported on the impact of 5-azacitidine or trichostatin A in the activation of EBV lytic phase in EBVaGC cell lines, leading to the lysis of tumor cells [157–160]. Despite the potential interest, it is important to clearly understand the mechanisms of EBV lytic phase activation using de-methylating agents.

3. Conclusions

EBV has been consistently associated with GC development for almost 30 years until in 2014, when The Cancer Genome Atlas Research Network recognized EBVaGC as a specific subtype of GC. Overall, EBVaGC represents almost 10% of all gastric cancer worldwide with a prevalence variation according to geographic and risk factors exposition. EBVaGC is more prevalent in males and younger patients and is frequently found in the proximal stomach. These tumors have a moderate to poor degree of differentiation and are characterized by a high content of CD8 + tumor-infiltrating lymphocytes and high expression of PD-L1 and PD-L2 and are therefore of great potential for immunotherapy. Indeed, EBVaGC seems to has a better prognosis and increased overall survival.

EBVaGC has distinctive molecular features: (1) extensive CpG island methylation (human and viral genomes) being described as EBV-CIMP (CpG island methylator phenotype); (2) mutations in *PIK3CA*, *ARID1A*, and *BCOR* genes; (3) amplification of 9p24.1 locus containing *JAK2*, *CD274*, *PDCD1LG2*, and *ERBB2*; (4) absence of *TP53* mutations; and (5) a microsatellite stable (MSs) phenotype. The development of therapeutical approaches directed to these specific molecular features (anti-PD1, PI3K/AKT/mTOR pathway inhibitors, or demethylating drugs) is expected to impact the GC management significantly.

In sum, EBVaGC is a specific subtype of GC, presenting special clinical and pathological characteristics that could be used for the development new potential therapeutical approaches, making this an important topic for the future of gastrointestinal tumors.

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