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].
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. pylori 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.](image-url)
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.
Epstein-Barr Virus - New Trends

6

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, 63, 66, 69, 70, 92–94]. 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 sug-
gested that it can be either by cell-to-cell with B-lymphocytes recruited in inflam-
matory 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 explana-
tion 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 establish-
ing 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 gas-
tric cancer are extremely controversial [106–109] and the majority report its pres-
ence 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 mecha-
nism 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 muta-
tions 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 addi-
tion, 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 infected gastric epithe-
lium 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.
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

| Latency I       | EBNA1 | EBNA2 | EBNA3 | LMP1 | LMP2 | EBERs | Malignancies                      |
|-----------------|-------|-------|-------|------|------|-------|-----------------------------------|
| Latency II      | x     |       | x     | x    | x    | x     | Nasopharyngeal carcinoma, Hodgkin lymphoma, T-cell non-Hodgkin lymphoma |
| Latency I-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 (ACP, 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]. Interestingly, 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 implementation 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 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 have 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 of new potential therapeutical approaches, making this an important topic for the future of gastrointestinal tumors.
Author details

Hugo Manuel Lopes de Sousa\textsuperscript{1,2*,} Joana Patrícia Costa Ribeiro\textsuperscript{3}
and Mafalda Basílio Timóteo\textsuperscript{2}

1 Virology Service, Portuguese Oncology Institute of Porto (IPO Porto),
Rua Dr. António Bernardino de Almeida, Porto, Portugal

2 Molecular Oncology and Viral Pathology Group (CI-IPOP), Portuguese Oncology Institute of Porto (IPO Porto), Rua Dr. António Bernardino de Almeida, Porto, Portugal

3 Molecular Haematology Department, Oxford University NHS Foundation Trust, Level 4, John Radcliffe Hospital, Oxford, UK

*Address all correspondence to: hugo.sousa@ipoporto.min-saude.pt

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
References

[1] Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0. Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr

[2] Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rossos S, Coebergh JW, Comber H, et al. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. European Journal of Cancer. 2013;49(6):1374-1403

[3] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2018;68(6):394-424

[4] RORENO. Registo Oncológico Nacional 2010. Porto: Instituto Português de Oncologia do Porto Francisco Gentil - EPE; 2016

[5] Fock KM. Review article: The epidemiology and prevention of gastric cancer. Alimentary Pharmacology & Therapeutics. 2014;40(3):250-260

[6] Balakrishnan M, George R, Sharma A, Graham DY. Changing trends in stomach cancer throughout the world. Current Gastroenterology Reports. 2017;19(8):36

[7] Ang TL, Fock KM. Clinical epidemiology of gastric cancer. Singapore Medical Journal. 2014;55(12):621-628

[8] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA: A Cancer Journal for Clinicians. 2015;65(2):87-108

[9] Collaborators GBDS. The global, regional, and national burden of stomach cancer in 195 countries, 1990-2017: A systematic analysis for the global burden of disease study 2017. The Lancet Gastroenterology & Hepatology. 2020;5(1):42-54

[10] Berth F, Bollschweiler E, Drebber U, Hoelscher AH, Moenig S. Pathohistological classification systems in gastric cancer: Diagnostic relevance and prognostic value. World Journal of Gastroenterology. 2014;20(19):5679-5684

[11] Van Cutsem E, Sagaert X, Topal B, Haustermans K, Prenen H. Gastric cancer. Lancet. 2016;388(10060):2654-2664

[12] Grundmann E, Schlake W. Histological classification of gastric cancer from initial to advanced stages. Pathology, Research and Practice. 1982;173(3):260-274

[13] Carneiro F, Seixas M, Sobrinho-Simoes M. New elements for an updated classification of the carcinomas of the stomach. Pathology, Research and Practice. 1995;191(6):571-584

[14] Goseki N, Takizawa T, Koike M. Differences in the mode of the extension of gastric cancer classified by histological type: New histological classification of gastric carcinoma. Gut. 1992;33(5):606-612

[15] Ming SC. Gastric carcinoma. A pathobiological classification. Cancer. 1977;39(6):2475-2485

[16] Cislo M, Filip AA, Arnold Offerhaus GJ, Cisel B, Rawicz-Pruszynski K, Skierucha M, et al. Distinct molecular subtypes of gastric cancer: From Lauren to molecular pathology. Oncotarget. 2018;9(27):19427-19442

[17] Cancer Genome Atlas Research N. Comprehensive
molecular characterization of gastric adenocarcinoma. Nature. 2014;513(7517):202-209

[18] Lauren P. The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathologica et Microbiologica Scandinavica. 1965;64:31-49

[19] Marques-Lespier JM, Gonzalez-Pons M, Cruz-Correa M. Current perspectives on gastric cancer. Gastroenterology Clinics of North America. 2016;45(3):413-428

[20] Kelley JR, Duggan JM. Gastric cancer epidemiology and risk factors. Journal of Clinical Epidemiology. 2003;56(1):1-9

[21] Bosman FT, Carneiro F, Hruban RH, Theise ND. WHO classification of tumours of the digestive system. In: World Health Organization Classification of Tumours. 4th edition. The International Agency for Research on Cancer; 2010

[22] Rocken C. Molecular classification of gastric cancer. Expert Review of Molecular Diagnostics. 2017;17(3):293-301. DOI: 10.1080/14737159.2017.1286985

[23] Sunakawa Y, Lenz HJ. Molecular classification of gastric adenocarcinoma: Translating new insights from the cancer genome atlas research network. Current Treatment Options in Oncology. 2015;16(4):17

[24] Ignatova E, Seriak D, Fedyanin M, Tryakin A, Pokataev I, Menshikova S, et al. Epstein-Barr virus-associated gastric cancer: Disease that requires special approach [published online ahead of print]. Gastric Cancer. 8 June 2020. DOI:10.1007/s10120-020-01095-z

[25] Liu Y, Sethi NS, Hinoue T, Schneider BG, Cherniack AD, Sanchez-Vega F, et al. Comparative molecular analysis of gastrointestinal adenocarcinomas. Cancer Cell. 2018;33(4):721-735 e8

[26] Sohn BH, Hwang JE, Jang HJ, Lee HS, Oh SC, Shim JJ, et al. Clinical significance of four molecular subtypes of gastric cancer identified by the Cancer Genome Atlas project [published online ahead of print]. Clinical Cancer Research. 26 July 2017. DOI: 10.1158/1078-0432.CCR-16-2211

[27] Kim Y, Cho MY, Kim J, Kim SN, Oh SC, Lee KA. Profiling cancer-associated genetic alterations and molecular classification of cancer in Korean gastric cancer patients. Oncotarget. 2017;8(41):69888-69905

[28] Cristescu R, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, et al. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. Nature Medicine. 2015;21(5):449-456

[29] Wang Q, Liu G, Hu C. Molecular classification of gastric adenocarcinoma. Gastroenterology Research. 2019;12(6):275-282

[30] Setia N, Agoston AT, Han HS, Mullen JT, Duda DG, Clark JW, et al. A protein and mRNA expression-based classification of gastric cancer. Modern Pathology. 2016;29(7):772-784

[31] Ahn S, Lee SJ, Kim Y, Kim A, Shin N, Choi KU, et al. High-throughput protein and mRNA expression-based classification of gastric cancers can identify clinically distinct subtypes, concordant with recent molecular classifications. The American Journal of Surgical Pathology. 2017;41(1):106-115

[32] Hu XT, He C. Recent progress in the study of methylated tumor suppressor genes in gastric cancer. Chinese Journal of Cancer. 2013;32(1):31-41

[33] Qiu T, Zhou X, Wang J, Du Y, Xu J, Huang Z, et al. MiR-145, miR-133a
and miR-133b inhibit proliferation, migration, invasion and cell cycle progression via targeting transcription factor Sp1 in gastric cancer. FEBS Letters. 2014;588(7):1168-1177

[34] Dinis-Ribeiro M, Areia M, de Vries AC, Marcos-Pinto R, Monteiro-Soares M, O’Connor A, et al. Management of precancerous conditions and lesions in the stomach (MAPS): Guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter Study Group (EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). Endoscopy. 2012;44(1):74-94

[35] Tan VP, Wong BC. Helicobacter pylori and gastritis: Untangling a complex relationship 27 years on. Journal of Gastroenterology and Hepatology. 2011;26(Suppl 1):42-45

[36] Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. The American Journal of Surgical Pathology. 1996;20(10):1161-1181

[37] Dixon MF. Gastrointestinal epithelial neoplasia: Vienna revisited. Gut. 2002;51(1):130-131

[38] Karimi P, Islami F, Anandasabapathy S, Freedman ND, Kamangar F. Gastric cancer: Descriptive epidemiology, risk factors, screening, and prevention. Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology. 2014;23(5):700-713

[39] de Martel C, Forman D, Plummer M. Gastric cancer: Epidemiology and risk factors. Gastroenterology Clinics of North America. 2013;42(2):219-240

[40] Goodenberger M, Lindor NM. Lynch syndrome and MYH-associated polyposis: Review and testing strategy. Journal of Clinical Gastroenterology. 2011;45(6):488-500

[41] Ramos M, Ribeiro Junior U, Viscondi JKY, Zilberstein B, Cecconello I, Eluf-Neto J. Risk factors associated with the development of gastric cancer—Case-control study. Revista da Associação Médica Brasileira (1992). 2018;64(7):611-619

[42] Fang X, Wei J, He X, An P, Wang H, Jiang L, et al. Landscape of dietary factors associated with risk of gastric cancer: A systematic review and dose-response meta-analysis of prospective cohort studies. European Journal of Cancer. 2015;51(18):2820-2832

[43] Ge S, Feng X, Shen L, Wei Z, Zhu Q, Sun J. Association between habitual dietary salt intake and risk of gastric cancer: A systematic review of observational studies. Gastroenterology Research and Practice. 2012;2012:808120

[44] Guggenheim DE, Shah MA. Gastric cancer epidemiology and risk factors. Journal of Surgical Oncology. 2013;107(3):230-236

[45] Yang P, Zhou Y, Chen B, Wan HW, Jia GQ, Bai HL, et al. Overweight, obesity and gastric cancer risk: Results from a meta-analysis of cohort studies. European Journal of Cancer. 2009;45(16):2867-2873

[46] Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. IARC Monogr Eval Carcinog Risks Hum. 1994;61:1-241

[47] Plummer M, Franceschi S, Vignat J, Forman D, de Martel C. Global burden
of gastric cancer attributable to *Helicobacter pylori*. International Journal of Cancer. 2015;136(2):487-490

[48] Gaddy JA, Radin JN, Loh JT, Zhang F, Washington MK, Peek RM Jr, et al. High dietary salt intake exacerbates *Helicobacter pylori*-induced gastric carcinogenesis. Infection and Immunity. 2013;81(6):2258-2267

[49] Mohammad AH, Assadian S, Couture F, Lefebvre KJ, El-Assaad W, Barres V, et al. V-ATPase-associated prorenin receptor is upregulated in prostate cancer after PTEN loss. Oncotarget. 2019;10(48):4923-4936

[50] Piazuelo MB, Epplein M, Correa P. Gastric cancer: An infectious disease. Infectious Disease Clinics of North America. 2010;24(4):853-869, vii

[51] Takada K. Epstein-Barr virus and gastric carcinoma. Molecular Pathology: MP. 2000;53(5):255-261

[52] Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. Nature Reviews. Cancer. 2004;22(33):5108-5121

[53] Javier RT, Butel JS. The history of tumor virology. Cancer Research. 2008;68(19):7693-7706

[54] Kutok JL, Wang F. Spectrum of Epstein-Barr virus-associated diseases. Annual Review of Pathology. 2006;1:375-404

[55] Murray PG, Young LS. Epstein-Barr virus infection: Basis of malignancy and potential for therapy. Expert Reviews in Molecular Medicine. 2001;3(28):1-20

[56] IWGotEoCRt H. Biological agents. Volume 100 B. A review of human carcinogens. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 2012;100(Pt B):1-441

[57] Proceedings of the IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Epstein-Barr virus and Kaposi’s sarcoma Herpesvirus/human Herpesvirus 8. Lyon, France, 17-24 June 1997. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 1997;70:1-492

[58] Naseem M, Barzi A, Brezden-Masley C, Puccini A, Berger MD, Tokunaga R, et al. Outlooks on Epstein-Barr virus associated gastric cancer. Cancer Treatment Reviews. 2018;66:15-22

[59] Tsao SW, Tsang CM, To KF, Lo KW. The role of Epstein-Barr virus in epithelial malignancies. The Journal of Pathology. 2015;235(2):323-333

[60] Young LS, Murray PG. Epstein-Barr virus and oncogenesis: From latent genes to tumours. Oncogene. 2003;22(33):5108-5121

[61] Burke AP, Yen TS, Shekitka KM, Sobin LH. Lymphoepithelial carcinoma of the stomach with Epstein-Barr virus demonstrated by polymerase chain reaction. Modern Pathology: An Official Journal of the United States and Canadian Academy of Pathology, Inc. 1990;3(3):377-380

[62] Shibata D, Weiss LM. Epstein-Barr virus-associated gastric adenocarcinoma. The American Journal of Pathology. 1992;140(4):769-774

[63] Shibata D, Tokunaga M, Uemura Y, Sato E, Tanaka S, Weiss LM. Association of Epstein-Barr virus with undifferentiated gastric carcinomas with intense lymphoid infiltration. Lymphoepithelioma-like carcinoma. The American Journal of Pathology. 1991;139(3):469-474

[64] Tokunaga M, Land CE, Uemura Y, Tokudome T, Tanaka S, Sato E. Epstein-Barr virus in gastric carcinoma.
The American Journal of Pathology. 1993;143(5):1250-1254

[65] Iizasa H, Nanbo A, Nishikawa J, Jinushi M, Yoshiyama H. Epstein-Barr virus (EBV)-associated gastric carcinoma. Viruses. 2012;4(12):3420-3439

[66] Lee JH, Kim SH, Han SH, An JS, Lee ES, Kim YS. Clinicopathological and molecular characteristics of Epstein-Barr virus-associated gastric carcinoma: A meta-analysis. Journal of Gastroenterology and Hepatology. 2009;24(3):354-365

[67] van Beek J, zur Hausen A, Klein Kranenburg E, van de Velde CJ, Middeldorp JM, van den Brule AJ, et al. EBV-positive gastric adenocarcinomas: A distinct clinicopathologic entity with a low frequency of lymph node involvement. Journal of Clinical Oncology. 2004;22(4):664-670

[68] Camargo MC, Murphy G, Koriyama C, Pfeiffer RM, Kim WH, Herrera-Goepfert R, et al. Determinants of Epstein-Barr virus-positive gastric cancer: An international pooled analysis. British Journal of Cancer. 2011;105(1):38-43

[69] Murphy G, Pfeiffer R, Camargo MC, Rabkin CS. Meta-analysis shows that prevalence of Epstein-Barr virus-positive gastric cancer differs based on sex and anatomic location. Gastroenterology. 2009;137(3):824-833

[70] Sousa H, Pinto-Correia AL, Medeiros R, Dinis-Ribeiro M. Epstein-Barr virus is associated with gastric carcinoma: The question is what is the significance? World Journal of Gastroenterology. 2008;14(27):4347-4351

[71] Oh JK, Weiderpass E. Infection and cancer: Global distribution and burden of diseases. Annals of Global Health. 2014;80(5):384-392

[72] Tavakoli A, Monavari SH, Solaymani Mohammadi F, Kiani SJ, Armat S, Farahmand M. Association between Epstein-Barr virus infection and gastric cancer: A systematic review and meta-analysis. BMC Cancer. 2020;20(1):493

[73] Bae JM, Kim EH. Epstein-Barr virus and gastric cancer risk: A meta-analysis with meta-regression of case-control studies. Journal of Preventive Medicine and Public Health. 2016;49(2):97-107

[74] Li S, Du H, Wang Z, Zhou L, Zhao X, Zeng Y. Meta-analysis of the relationship between Epstein-Barr virus infection and clinicopathological features of patients with gastric carcinoma. Science China. Life Sciences. 2010;53(4):524-530

[75] Ribeiro J, Oliveira A, Malta M, Oliveira C, Silva F, Galaghvar A, et al. Clinical and pathological characterization of Epstein-Barr virus-associated gastric carcinomas in Portugal. World Journal of Gastroenterology. 2017;23(40):7292-7302

[76] Boysen T, Friborg J, Strubolt K, Hamilton-Dutoit S, Goertz S, Wohlfahrt J, et al. Epstein-Barr virus-associated gastric carcinoma among patients with pernicious anemia. International Journal of Cancer. 2011;129(11):2756-2760

[77] Kim RH, Chang MS, Kim HJ, Song KS, Kim YS, Choi BY, et al. Medical history and lifestyle factors contributing to Epstein-Barr virus-associated gastric carcinoma and conventional gastric carcinoma in Korea. Anticancer Research. 2010;30(6):2469-2475

[78] Sukawa Y, Yamamoto H, Nosho K, Kunimoto H, Suzuki H, Adachi Y, et al. Alterations in the human epidermal growth factor receptor 2-phosphatidylinositol 3-kinase-v-Akt pathway in gastric cancer.
World Journal of Gastroenterology. 2012;18(45):6577-6586

[79] Truong CD, Feng W, Li W, Khoury T, Li Q, Alrawi S, et al. Characteristics of Epstein-Barr virus-associated gastric cancer: A study of 235 cases at a comprehensive cancer center in U.S.A. Journal of Experimental & Clinical Cancer Research: CR. 2009;28

[80] Camargo MC, Kim WH, Chiaramalli AM, Kim KM, Corvalan AH, Matsuo K, et al. Improved survival of gastric cancer with tumour Epstein-Barr virus positivity: An international pooled analysis. Gut. 2014;63(2):236-243

[81] Rymbai ML, Ramalingam VV, Samarasan I, Chandran BS, Mathew G, Jerobin J, et al. Frequency of Epstein-Barr virus infection as detected by messenger RNA for EBNA 1 in histologically proven gastric adenocarcinoma in patients presenting to a tertiary care center in South India. Indian Journal of Medical Microbiology. 2015;33(3):369-373

[82] Morales-Sanchez A, Fuentes-Panana EM. Epstein-Barr virus-associated gastric cancer and potential mechanisms of oncogenesis. Current Cancer Drug Targets. 2017;17(6):534-554

[83] Jacome AA, Lima EM, Kazzi AI, Chaves GF, Mendonca DC, Maciel MM, et al. Epstein-Barr virus-positive gastric cancer: A distinct molecular subtype of the disease? Revista da Sociedade Brasileira de Medicina Tropical. 2016;49(2):150-157

[84] Qiu K, Tomita Y, Hashimoto M, Ohsawa M, Kawano K, Wu DM, et al. Epstein-Barr virus in gastric carcinoma in Suzhou, China and Osaka, Japan: Association with clinico-pathologic factors and HLA-subtype. International Journal of Cancer. 1997;71(2):155-158

[85] Park JH, Kim EK, Kim YH, Kim JH, Bae YS, Lee YC, et al. Epstein-Barr virus positivity, not mismatch repair-deficiency, is a favorable risk factor for lymph node metastasis in submucosa-invasive early gastric cancer. Gastric Cancer. 2016;19(4):1041-1051

[86] Rickinson AB. Co-infections, inflammation and oncogenesis: Future directions for EBV research. Seminars in Cancer Biology. 2014;26:99-115

[87] Murai K, Kakushima N, Sugino T, Yoshida M, Kawata N, Tanaka M, et al. Epstein-Barr virus positivity among surgically resected intramucosal gastric cancer. Digestive Endoscopy: Official Journal of the Japan Gastroenterological Endoscopy Society. 2018;30(5):667-671

[88] Corvalan A, Koriyama C, Akiba S, Eizuru Y, Backhouse C, Palma M, et al. Epstein-Barr virus in gastric carcinoma is associated with location in the cardia and with a diffuse histology: A study in one area of Chile. International Journal of Cancer. 2001;94(4):527-530

[89] Huh CW, Jung DH, Kim H, Kim H, Youn YH, Park H, et al. Clinicopathologic features of gastric carcinoma with lymphoid stroma in early gastric cancer. Journal of Surgical Oncology. 2016;114(6):769-772

[90] Lim H, Park YS, Lee JH, Son DH, Ahn JY, Choi KS, et al. Features of gastric carcinoma with lymphoid stroma associated with Epstein-Barr virus. Clinical Gastroenterology and Hepatology: The Official Clinical Practice Journal of the American Gastroenterological Association. 2015;13(10):1738-1744 e2

[91] Shinozaki-Ushiku A, Kunita A, Fukayama M. Update on Epstein-Barr virus and gastric cancer (review). International Journal of Oncology. 2015;46(4):1421-1434

[92] Watanabe H, Enjoji M, Imai T. Gastric carcinoma with lymphoid stroma. Its morphologic characteristics

[93] Shinozaki-Ushiku A, Kunita A, Fukayama M. Update on Epstein-Barr virus and gastric cancer (review). International Journal of Oncology. 2015;46(4):1421-1434
and prognostic correlations. Cancer. 1976;38(1):232-243

[93] Lim H, Lee IS, Lee JH, Park YS, Kang HJ, Na HK, et al. Clinical application of early gastric carcinoma with lymphoid stroma based on lymph node metastasis status. Gastric Cancer. 2017;20(5):793-801. DOI: 10.1007/s10120-017-0703-z

[94] Wang ZH, Zhao JJ, Yuan Z. Lymphoepithelioma-like gastric carcinoma: A case report and review of the literature. World Journal of Gastroenterology. 2016;22(10):3056-3061

[95] Carrasco-Avino G, Riquelme I, Padilla O, Villaseca M, Aguayo FR, Corvalan AH. The conundrum of the Epstein-Barr virus-associated gastric carcinoma in the Americas. Oncotarget. 2017;8(43):75687-75698

[96] Chang MS, Lee HS, Kim CW, Kim YI, Kim WH. Clinicopathologic characteristics of Epstein-Barr virus-incorporated gastric cancers in Korea. Pathology, Research and Practice. 2001;197(6):395-400

[97] Yoshiwara E, Koriyama C, Akiba S, Itoh T, Minakami Y, Chirinos JL, et al. Epstein-Barr virus-associated gastric carcinoma in Lima, Peru. Journal of Experimental & Clinical Cancer Research: CR. 2005;24(1):49-54

[98] Ragazzi M, Ciarrocchi A, Sancisi V, Gandolfi G, Bisagni A, Piana S. Update on anaplastic thyroid carcinoma: Morphological, molecular, and genetic features of the most aggressive thyroid cancer. International Journal of Endocrinology. 2014;2014:790834

[99] Nishikawa J, Iizasa H, Yoshiyama H, Shimokuri K, Kobayashi Y, Sasaki S, et al. Clinical importance of Epstein(-)Barr virus-associated gastric cancer. Cancers (Basel). 29 May 2018;10(6):167. DOI: 10.3390/cancers10060167

[100] Young LS, Yap LF, Murray PG. Epstein-Barr virus: More than 50 years old and still providing surprises. Nature Reviews Cancer. 2016;16(12):789-802

[101] Nemerow GR, Houghten RA, Moore MD, Cooper NR. Identification of an epitope in the major envelope protein of Epstein-Barr virus that mediates viral binding to the B lymphocyte EBV receptor (CR2). Cell. 1989;56(3):369-377

[102] Nemerow GR, Mold C, Schwend VK, Tollefson V, Cooper NR. Identification of gp350 as the viral glycoprotein mediating attachment of Epstein-Barr virus (EBV) to the EBV/C3d receptor of B cells: Sequence homology of gp350 and C3 complement fragment C3d. Journal of Virology. 1987;61(5):1416-1420

[103] Yue W, Zhu M, Zuo L, Xin S, Zhang J, Liu L, et al. Early pattern of Epstein-Barr virus infection in gastric epithelial cells by "cell-in-cell". Virologica Sinica. 2019;34(3):253-261

[104] Ohnishi N, Yuasa H, Tanaka S, Sawa H, Miura M, Matsui A, et al. Transgenic expression of Helicobacter pylori CagA induces gastrointestinal and hematopoietic neoplasms in mouse. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(3):1003-1008

[105] Martinez-Lopez JL, Torres J, Camorlinga-Ponce M, Mantilla A, Leal YA, Fuentes-Panana EM. Evidence of Epstein-Barr virus association with gastric cancer and non-atrophic gastritis. Viruses. 2014;6(1):301-318

[106] Gulley ML, Pulitzer DR, Eagan PA, Schneider BG. Epstein-Barr virus infection is an early event in gastric carcinogenesis and is independent of bcl-2 expression and p53 accumulation. Human Pathology. 1996;27(1):20-27

[107] Hungermann D, Muller S, Spieker T, Lisner R, Niedobitek G,
Herbst H. Low prevalence of latently Epstein-Barr virus-infected cells in chronic gastritis. Microscopy Research and Technique. 2001;53(6):409-413

[108] Nogueira C, Mota M, Gradiz R, Cipriano MA, Caramelo F, Cruz H, et al. Prevalence and characteristics of Epstein–Barr virus-associated gastric carcinomas in Portugal. Infectious Agents and Cancer. 2017;12(41):1-8

[109] Zur Hausen A, van Rees BP, van Beek J, Craanen ME, Bloemena E, Offerhaus GJ, et al. Epstein-Barr virus in gastric carcinomas and gastric stump carcinomas: A late event in gastric carcinogenesis. Journal of Clinical Pathology. 2004;57(5):487-491

[110] de Souza CR, de Oliveira KS, Ferraz JJ, Leal MF, Calcagnno DQ, Seabra AD, et al. Occurrence of Helicobacter pylori and Epstein-Barr virus infection in endoscopic and gastric cancer patients from northern Brazil. BMC Gastroenterology. 2014;14:179

[111] Yanai H, Takada K, Shimizu N, Mizugaki Y, Tada M, Okita K. Epstein-Barr virus infection in non-carcinomatous gastric epithelium. The Journal of Pathology. 1997;183(3):293-298

[112] Luqmani YA, Linjawi SO, Shousha S. Detection of Epstein-Barr virus in gastrectomy specimens. Oncology Reports. 2001;8(5):995-999

[113] Hirano A, Yanai H, Shimizu N, Okamoto T, Matsubara Y, Yamamoto K, et al. Evaluation of epstein-barr virus DNA load in gastric mucosa with chronic atrophic gastritis using a real-time quantitative PCR assay. International Journal of Gastrointestinal Cancer. 2003;34(2-3):87-94

[114] Chen ZM, Shah R, Zuckerman GR, Wang HL. Epstein-Barr virus gastritis: An underrecognized form of severe gastritis simulating gastric lymphoma.

The American Journal of Surgical Pathology. 2007;31(9):1446-1451

[115] Tsang CM, Yip YL, Lo KW, Deng W, To KF, Hau PM, et al. Cyclin D1 overexpression supports stable EBV infection in nasopharyngeal epithelial cells. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(50):E3473-E3482

[116] Tsang CM, Zhang G, Seto E, Takada K, Deng W, Yip YL, et al. Epstein-Barr virus infection in immortalized nasopharyngeal epithelial cells: Regulation of infection and phenotypic characterization. International Journal of Cancer. 2010;127(7):1570-1583

[117] Abe H, Kaneda A, Fukayama M. Epstein-Barr virus-associated gastric carcinoma: Use of host cell machineries and somatic gene mutations. Pathobiology: Journal of Immunopathology, Molecular and Cellular Biology. 2015;82(5):212-223

[118] Minoura-Etoh J, Gotoh K, Sato R, Ogata M, Kaku N, Fujioka T, et al. Helicobacter pylori-associated oxidant monochloramine induces reactivation of Epstein-Barr virus (EBV) in gastric epithelial cells latently infected with EBV. Journal of Medical Microbiology 2006;55(Pt 7):905-911.

[119] Herrera-Goepfert R, Akiba S, Koriyama C, Ding S, Reyes E, Itoh T, et al. Epstein-Barr virus-associated gastric carcinoma: Evidence of age-dependence among a Mexican population. World Journal of Gastroenterology. 2005;11(39):6096-6103

[120] Galetsky SA, Tsvetnov VV, Land CE, Afanasieva TA, Petrovichev NN, Gurtsevitch VE, et al. Epstein-Barr-virus-associated gastric cancer in Russia. International Journal of Cancer. 1997;73(6):786-789
[121] Nogueira Tde B, Artigiani RN, Herani BF, Waisberg J. *H. pylori* infection, endoscopic, histological aspects and cell proliferation in the gastric mucosa of patients submitted to roux-en-Y gastric bypass with contention ring: A cross sectional endoscopic and immunohistochemical study. Arquivos de Gastroenterologia. 2016;53(1):55-60

[122] Camargo MC, Kim KM, Matsuo K, Torres J, Liao LM, Morgan DR, et al. Anti-*Helicobacter pylori* antibody profiles in Epstein-Barr virus (EBV)-positive and EBV-negative gastric cancer. Helicobacter. 2016;21(2):153-157

[123] Matsusaka K, Funata S, Fukayama M, Kaneda A. DNA methylation in gastric cancer, related to *Helicobacter pylori* and Epstein-Barr virus. World Journal of Gastroenterology. 2014;20(14):3916-3926

[124] White MK, Pagano JS, Khalili K. Viruses and human cancers: A long road of discovery of molecular paradigms. Clinical Microbiology Reviews. 2014;27(3):463-481

[125] Raab-Traub N. EBV-induced oncogenesis. In: Arvin A, Campadelli-Fiume G, Mocarski E, Moore PS, Roizman B, Whitley R, et al., editors. Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis. Cambridge: Cambridge University Press; 2007. Chapter 55. Available from: https://www.ncbi.nlm.nih.gov/books/NBK47429/

[126] Tang W, Morgan DR, Meyers MO, Dominguez RL, Martinez E, Kakudo K, et al. Epstein-Barr virus infected gastric adenocarcinoma expresses latent and lytic viral transcripts and has a distinct human gene expression profile. Infectious Agents and Cancer. 2012;7(1):21

[127] Ribeiro J, Oliveira C, Malta M, Sousa H. Epstein-Barr virus gene expression and latency pattern in gastric carcinomas: A systematic review. Future Oncology. 2017;13(6):567-579

[128] Amon W, Farrell PJ. Reactivation of Epstein-Barr virus from latency. Reviews in Medical Virology. 2005;15(3):149-156

[129] Raab-Traub N. Novel mechanisms of EBV-induced oncogenesis. Current Opinion in Virology. 2012;2(4):453-458

[130] Fitzsimmons L, Kelly GL. EBV and apoptosis: The viral master regulator of cell fate? Viruses. 13 Nov 2017;9(11):339. DOI: 10.3390/v9110339

[131] Curran JA, Laverty FS, Campbell D, Macdiarmid J, Wilson JB. Epstein-Barr virus encoded latent membrane protein-1 induces epithelial cell proliferation and sensitizes transgenic mice to chemical carcinogenesis. Cancer Research. 2001;61(18):6730-6738

[132] Yau TO, Tang CM, Yu J. Epigenetic dysregulation in Epstein-Barr virus-associated gastric carcinoma: Disease and treatments. World Journal of Gastroenterology. 2014;20(21):6448-6456

[133] Zouridis H, Deng N, Ivanova T, Zhu Y, Wong B, Huang D, et al. Methylation subtypes and large-scale epigenetic alterations in gastric cancer. Science Translational Medicine. 2012;4(156):156ra40

[134] Gulley ML. Genomic assays for Epstein-Barr virus-positive gastric adenocarcinoma. Experimental & Molecular Medicine. 2015;47:e134

[135] Chapel F, Fabiani B, Davi F, Raphael M, Tepper M, Champault G, et al. Epstein-Barr virus and gastric carcinoma in Western patients: Comparison of pathological parameters and p53 expression in EBV-positive and negative tumours. Histopathology. 2000;36(3):252-261
[136] Sun Q, Brewer N, Dunham K, Chen L, Bao L, Burton R, et al. Interferon-gamma expressing EBV LMP2A-specific T cells for cellular immunotherapy. Cellular Immunology. 2007;246(2):81-91

[137] Samuels Y, Waldman T. Oncogenic mutations of PIK3CA in human cancers. Current Topics in Microbiology and Immunology. 2010;347:21-41

[138] Ribeiro J, Malta M, Galaghar A, Silva F, Afonso LP, Medeiros R, et al. P53 deregulation in Epstein-Barr virus-associated gastric cancer. Cancer Letters. 2017;404:37-43

[139] Derks S, Liao X, Chiaravalli AM, Xu X, Camargo MC, Solcia E, et al. Abundant PD-L1 expression in Epstein-Barr virus-infected gastric cancers. Oncotarget. 2016;7(22):32925-32932

[140] Koh J, Ock CY, Kim JW, Nam SK, Kwak Y, Yun S, et al. Clinicopathologic implications of immune classification by PD-L1 expression and CD8-positive tumor-infiltrating lymphocytes in stage II and III gastric cancer patients. Oncotarget. 2017;8(16):26356-26367

[141] Kang BW, Baek DW, Kang H, Baek JH, Kim JG. Novel therapeutic approaches for Epstein-Barr virus associated gastric cancer. Anticancer Research. 2019;39(8):4003-4010

[142] Kim ST, Cristescu R, Bass AJ, Kim KM, Odegaard JJ, Kim K, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. Nature Medicine. 2018;24(9):1449-1458

[143] Gu L, Chen M, Guo D, Zhu H, Zhang W, Pan J, et al. PD-L1 and gastric cancer prognosis: A systematic review and meta-analysis. PLoS One. 2017;12(8):e0182692

[144] Fuchs CS, Doi T, Jang RW, Muro K, Satoh T, Machado M, et al. Safety and efficacy of pembrolizumab monotherapy in patients with previously treated advanced gastric and gastroesophageal junction cancer: Phase 2 clinical KEYNOTE-059 trial. JAMA Oncology. 2018;4(5):e180013

[145] Muro K, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, et al. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): A multicentre, open-label, phase 1b trial. The Lancet Oncology. 2016;17(6):717-726

[146] Joshi SS, Maron SB, Catenacci DV. Pembrolizumab for treatment of advanced gastric and gastroesophageal junction adenocarcinoma. Future Oncology. 2018;14(5):417-430

[147] Kwong YL, Chan TSY, Tan D, Kim SJ, Poon LM, Mow B, et al. PD1 blockade with pembrolizumab is highly effective in relapsed or refractory NK/T-cell lymphoma failing l-asparaginase. Blood. 2017;129(17):2437-2442

[148] Moehler M, Ryu MH, Dvorkin M, Lee KW, Coskun HS, Wong R, et al. Maintenance avelumab versus continuation of first-line chemotherapy in gastric cancer: JAVELIN Gastric 100 study design. Future Oncology. 2019;15(6):567-577

[149] Bang YJ, Ruiz EY, Van Cutsem E, Lee KW, Wyrwicz L, Schenker M, et al. Phase III, randomised trial of avelumab versus physician’s choice of chemotherapy as third-line treatment of patients with advanced gastric or gastro-oesophageal junction cancer: Primary analysis of JAVELIN Gastric 300. Annals of Oncology. 2018;29(10):2052-2060

[150] Panda A, Mehnert JM, Hirshfield KM, Riedlinger G, Damare S, Saunders T, et al. Immune activation and benefit from avelumab in EBV-positive gastric cancer. Journal of the National Cancer Institute. 2018;110(3):316-320
[151] Diaz-Serrano A, Angulo B, Dominguez C, Pazo-Cid R, Salud A, Jimenez-Fonseca P, et al. Genomic profiling of HER2-positive gastric cancer: PI3K/Akt/mTOR pathway as predictor of outcomes in HER2-positive advanced gastric cancer treated with trastuzumab. The Oncologist. 2018;23(9):1092-1102

[152] Fang WL, Huang KH, Lan YT, Lin CH, Chang SC, Chen MH, et al. Mutations in PI3K/AKT pathway genes and amplifications of PIK3CA are associated with patterns of recurrence in gastric cancers. Oncotarget. 2016;7(5):6201-6220

[153] Ito C, Nishizuka SS, Ishida K, Uesugi N, Sugai T, Tamura G, et al. Analysis of PIK3CA mutations and PI3K pathway proteins in advanced gastric cancer. The Journal of Surgical Research. 2017;212:195-204

[154] Owonikoko TK, Harvey RD, Carthon B, Chen Z, Lewis C, Collins H, et al. A phase I study of safety, pharmacokinetics, and pharmacodynamics of concurrent everolimus and buparlisib treatment in advanced solid tumors. Clinical Cancer Research. 2020;26(11):2497-2505

[155] Rodon J, Brana I, Siu LL, De Jonge MJ, Homji N, Mills D, et al. Phase I dose-escalation and -expansion study of buparlisib (BKM120), an oral pan-class I PI3K inhibitor, in patients with advanced solid tumors. Investigational New Drugs. 2014;32(4):670-681

[156] Kim KJ, Kim JW, Sung JH, Suh KJ, Lee JY, Kim SH, et al. PI3K-targeting strategy using alpelisib to enhance the antitumor effect of paclitaxel in human gastric cancer. Scientific Reports. 2020;10(1):12308

[157] Jung EJ, Lee YM, Lee BL, Chang MS, Kim WH. Lytic induction and apoptosis of Epstein-Barr virus-associated gastric cancer cell line with epigenetic modifiers and ganciclovir. Cancer Letters. 2007;247(1):77-83

[158] Chang MS, Uozaki H, Chong JM, Ushiku T, Sakuma K, Ishikawa S, et al. CpG island methylation status in gastric carcinoma with and without infection of Epstein-Barr virus. Clinical Cancer Research. 2006;12(10):2995-3002

[159] Schneider BJ, Shah MA, Klute K, Ocean A, Popa E, Alterki N, et al. Phase I study of epigenetic priming with azacitidine prior to standard neoadjuvant chemotherapy for patients with resectable gastric and esophageal adenocarcinoma: Evidence of tumor hypomethylation as an indicator of major histopathologic response. Clinical Cancer Research. 2017;23(11):2673-2680

[160] Kusano M, Toyota M, Suzuki H, Akino K, Aoki F, Fujita M, et al. Genetic, epigenetic, and clinicopathologic features of gastric carcinomas with the CpG island methylator phenotype and an association with Epstein-Barr virus. Cancer. 2006;106(7):1467-1479